Can we re-invent feeding animals? Feed companies? Ingredients and nutrient supplies? Can we change how food animal products and crops are perceived by, and marketed to, consumers?

The theme of the 20th Annual Alltech Symposium focuses on doing exactly that: re-defining how we feed animals and re-imagining our agribusinesses to create the compelling force behind an exciting future.

The basic and applied research is done. Natural feeding strategies can reduce environmental impact, replace antibiotics, make producers more competitive and change how consumers view and value food animal products. There is a shift in the dynamic of the industry as well. Change is no longer something ‘happening to’ the feed industry. Change is something the industry makes happen.
Nutritional Biotechnology in the Feed and Food Industries
Nutritional Biotechnology in the Feed and Food Industries

Proceedings of
Alltech’s Twentieth Annual Symposium

Edited by TP Lyons and KA Jacques
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2Plant Protection Department, Faculty of Agriculture, Ege University, Izmir, Turkey

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Re-imagining the feed industry: focus on price, perception and policy

T. PEARSE LYONS

Alltech Inc., Nicholasville, Kentucky, USA

Global agricultural is in turmoil. World trade barriers are coming down. The EU Common Agricultural Policy and the US supports may become a thing of the past, opening up markets and leveling the playing field. BSE cases in Canada and the US have thrown these industries into a quandary, while southeast Asia is reeling from the impact of avian flu.

Natural feed technologies such as those offered by Alltech have never been more in favor. Increasing acceptance of natural feeding strategies reflects the realization that there is no going back to previous methods in today’s consumer-oriented markets. At a recent roundtable discussion sponsored by Alltech in the aquaculture sector, one attendee described the event as “the most exciting discussion in which I have participated in the past 10 years”. Such is the enthusiasm in these markets. We believe that every cloud has a silver lining, but for agriculture the difficult times of these past years can only be turned around if we embrace change and recognize the three key determinants of success: Price competitiveness, Perception of the consumer, and Policies we can depend on to guide us now and in the future.

Success Factor No. 1: Price competitiveness

How can other markets compete given the size of US and Brazilian farms? How can a small country maintain its position? To be successful we must adopt new technologies, which is how Brazil made such remarkable strides forward in a comparatively short period of time.

What are some of the new technologies that can make food animal production more price-competitive? The two most important are technologies that improve the efficiency with which we use feed ingredient raw materials, and the other is through improved animal health.

<table>
<thead>
<tr>
<th>Success Factor 1</th>
<th>Lowering production costs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw materials</td>
<td>Utilize wider availability of cereals, protein sources</td>
</tr>
<tr>
<td>Improved</td>
<td>New generation SSF enzymes to improve efficiency</td>
</tr>
<tr>
<td>Animal health</td>
<td>Herd longevity: cows, sows</td>
</tr>
<tr>
<td></td>
<td>More piglets weaned</td>
</tr>
<tr>
<td></td>
<td>More high quality chicks per breeder</td>
</tr>
<tr>
<td></td>
<td>Reduce health costs</td>
</tr>
</tbody>
</table>

THE KOJI PROCESS OF SOLID STATE FERMENTATION: LOWERING THE COST OF CONVERTING FEED TO MEAT AND EGGS

Nature ensures utilization of its abundant feedstuffs by placing microbes and animals in symbiosis. In both ruminants and monogastrics, rumen or hindgut microbes digest the structural carbohydrates in fiber to release energy and ultimately to provide protein from microbial cells for animal use. Without the microbe’s ability to produce enzyme arrays, the host animal could not make much use of a vegetable-based diet.

Alltech harnessed this symbiosis with a unique fermented koji, which is sold under the name of Vegpro™ SSF. In the koji fermentation the enzyme-producing microbes are grown on substrates similar to those that food animals consume, which induces the microbe to produce the spectrum of enzymes most appropriate for the job. When added to poultry and pig diets containing oilseed meals such as soya and
canola, seven enzyme activities in Vegpro™ SSF interact to boost release of energy and protein. Brazilian experience in formulating poultry and pig diets with Vegpro™ SSF has shown that savings of as much as $10-15 per tonne are possible. As global reliance on shorter supplies of vegetable proteins increases, this technology could be crucial (Table 1). Why? Because the enzymes release the energy in soya and improve protein digestibility. A summary of experience with Vegpro™ SSF in South America is found in Table 2.

Table 1. The soybean challenge: usage and production growing at different rates.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Production</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>2,890</td>
<td>78.6</td>
<td>-11</td>
</tr>
<tr>
<td>Brazil</td>
<td>1,598</td>
<td>43.5</td>
<td>+10</td>
</tr>
<tr>
<td>Argentina</td>
<td>1,084</td>
<td>29.5</td>
<td>+12</td>
</tr>
<tr>
<td>China</td>
<td>556</td>
<td>15.1</td>
<td>+9</td>
</tr>
<tr>
<td>Total</td>
<td>6,751</td>
<td>183.7</td>
<td>+6</td>
</tr>
<tr>
<td>Use projection for 2004-2005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>China</td>
<td></td>
<td></td>
<td>+15%</td>
</tr>
<tr>
<td>Russia</td>
<td></td>
<td></td>
<td>+25%</td>
</tr>
</tbody>
</table>

LOWER PRODUCTION COSTS BY IMPROVING ANIMAL HEALTH

At a recent presentation on the future of American dairy farming, Dr. Steve Koenig pointed out that animal health is the key to success in the future. While he used the dairy farm to illustrate his points, they could be applied to any animal production system. An example illustrating the impact improved animal health has on productivity is cow longevity. Dairy cows average only two lactations in several regions of the US, while 60% of all sows are culled after only three parities. Given that peak milk production in the dairy cow and peak sow productivity are well after these ages, the amount of lost production is astounding. At an average of 10,000 kg of milk per lactation and 20,000 per lifetime, this means a replacement cost of $0.06/kg of milk - nearly 24% of the total selling price of milk! Imagine any company devoting 20-25% of the sales to replacing the equipment! They could not survive, and nor can we. If an extra lactation can be achieved, replacement cost drops to $0.04/kg or 17% of the total cost (Table 3).

Table 3. Calculated cost of replacing a cow based on two or three lactations.

<table>
<thead>
<tr>
<th>Replacement heifer cost, USD</th>
<th>1400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culled cow price, USD</td>
<td>200</td>
</tr>
<tr>
<td>Milk per lactation, kg</td>
<td>10,000</td>
</tr>
<tr>
<td>Milk price, USD/kg</td>
<td>0.20</td>
</tr>
<tr>
<td>Replacement cost of the cow, USD/kg milk</td>
<td></td>
</tr>
<tr>
<td>Longevity: 2 lactations</td>
<td>0.06</td>
</tr>
<tr>
<td>Longevity: 3 lactations</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Sel-Plex® impact on health

Cows and sows are culled for reasons of health and reproduction, both of which are at risk when selenium status is marginal; and it is this specific area where Sel-Plex®, organic selenium produced by yeast, can help. Selenium in Sel-Plex® is present in the ideal ratio of selenoamino acids. When mastitis/MMA impact is reduced, and selenium needs for reproduction are met, commercial experience with Sel-Plex is that herd longevity can be increased, however an extra lactation is just one of the benefits noted.

Sel-Plex® has implications for health and reproductive efficiency in all food animal species (Table 4). For sows, commercial and university reports have indicated more pigs born alive and more pigs weaned; and a review of poultry data in refereed publications alone demonstrates increased number of chicks hatched (2-4) per broiler breeder hen. Furthermore, improvements in health make the switch to Sel-Plex® easy. Is this new? No. Dr. Don Mahan...
T.P. Lyons 3

at Ohio State predicted this in 1995! The issue is as ever – not whether the new technology will lower costs – it can – but whether the will to make the change in order to reap the benefits exists. Organic selenium – Sel-Plex® – has truly redefined selenium nutrition and indeed vitamin E and ‘antioxidant’ supplementation in general. However, there can be no half measures. Full replacement of sodium selenite at all stages of life is required. Health is a lifelong requirement.

Table 4. Sel-Plex® impact on herd health and productivity.

<table>
<thead>
<tr>
<th>Category</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy</td>
<td>Cow longevity in the herd: lifetime yield</td>
</tr>
<tr>
<td></td>
<td>Fewer days open</td>
</tr>
<tr>
<td></td>
<td>Fewer services/conception</td>
</tr>
<tr>
<td></td>
<td>No Se injections needed</td>
</tr>
<tr>
<td></td>
<td>Reduced retained placenta incidence</td>
</tr>
<tr>
<td></td>
<td>Reduced mastitis incidence/impact</td>
</tr>
<tr>
<td></td>
<td>Lower SCC</td>
</tr>
<tr>
<td></td>
<td>Calf livability</td>
</tr>
<tr>
<td></td>
<td>Supranutritional vitamin E levels unneeded</td>
</tr>
<tr>
<td>Beef</td>
<td>Meat quality</td>
</tr>
<tr>
<td></td>
<td>Calf livability</td>
</tr>
<tr>
<td></td>
<td>Feed efficiency</td>
</tr>
<tr>
<td>Pigs</td>
<td>Sow longevity in the herd: lifetime productivity</td>
</tr>
<tr>
<td></td>
<td>An extra 0.5-1 pigs weaned/litter</td>
</tr>
<tr>
<td></td>
<td>Reduced MMA</td>
</tr>
<tr>
<td></td>
<td>Piglet health at birth and weaning</td>
</tr>
<tr>
<td>Chickens</td>
<td>Breeders</td>
</tr>
<tr>
<td></td>
<td>More settable eggs produced</td>
</tr>
<tr>
<td></td>
<td>Fertility (male and female)</td>
</tr>
<tr>
<td></td>
<td>Hatchability</td>
</tr>
<tr>
<td></td>
<td>More chicks per hen</td>
</tr>
<tr>
<td>Broilers</td>
<td>Reduced mortality/culls</td>
</tr>
<tr>
<td></td>
<td>Improved uniformity</td>
</tr>
<tr>
<td></td>
<td>Feed efficiency</td>
</tr>
<tr>
<td></td>
<td>Meat quality</td>
</tr>
<tr>
<td>Layers</td>
<td>Egg production</td>
</tr>
<tr>
<td></td>
<td>Egg quality</td>
</tr>
<tr>
<td></td>
<td>Shell quality</td>
</tr>
</tbody>
</table>

Knowledge about organic selenium is accumulating at an incredible rate in all disciplines, but agriculture is the sector able to take greatest advantage of it. Still, it is always best to remember how much we still do not know! Not long ago science only knew of the role of selenium in glutathione peroxidase (GSH-Px). Now we know there are six forms of GSH-Px, and 30-50 selenoproteins. Likewise, we now know that there are a wide range of selenocompounds in plants and yeast, and failure to discount the importance of any of them because we do not today know their function would be absurd. Nature rarely makes things for no reason. Modern analytical techniques have revealed one reason response differs between selenium yeast sources. French researchers noted that the profile of selenium compounds differs among commercial selenium sources (Figure 1). Reasons might include differing growth media, pH and temperature conditions and(or) yeast strain. As such, data generated from a product manufactured by one process cannot be extrapolated to another. This why in clearing ‘selenium yeast’ for use in the US following review of Sel-Plex®, FDA defined an allowable product as one made precisely by this process. In effect, the regulators are holding all new products to the standard set by Sel-Plex®.

Key Success Factor No. 2: Perceptions of the consumer

Overcoming the negative perception of the consumer is more difficult to achieve than a reduction in costs. Due to a litany of scares – BSE, Foot and Mouth Disease and dioxin contaminations – the public is often suspicious of modern agriculture.

Success Factor 2
Changing consumer perceptions

Animal feed contains antibiotics used in human medicine
Animal feeds contain recycled ‘dangerous’ animal proteins
Agriculture pollutes soil and water
Meat, milk and eggs are not ‘healthy’ foods

The recent mad cow scare in the US illustrates how reluctant as an industry we are to change, and perhaps validates consumer skepticism and the demand for greater scrutiny. Carol Tucker Forman, director of the Food Policy Institute of the Consumer Federation of America, was quoted as saying “the damage to the American meat industry, and therefore the feed industry, costs infinitely more than anything US cattlemen would have to pay to do things right”. But doing things ‘right’ is not something we are always perceived to excel at. Least cost formulations occasionally overrule common sense, and it seems incredible that in a time when markets are asking for total transparency and traceability that one would leave anything to chance, much less take unnecessary risks. As we marvel at the apparent “repeating of the European BSE mistakes” in the US, we remind ourselves that the perception is that many of our problems originate from what and how we feed.
livestock. This should not be the case; there are a number of alternatives in use in all sectors of the industry. Let’s briefly evaluate ways in which natural feeding programs combat perceptions of food animal agriculture.

PERCEPTION 1: ALL ANIMAL FEED CONTAINS GROWTH-PROMOTING ANTIBIOTICS

Less true each year. While there were never antibiotics in ‘all’ feeds, even those sectors where inclusion was routine such as grow-finish pigs and broiler diets are steadily eliminating AGPs and have replaced them with natural products and programs that promote health and growth.

Bio-Mos® was introduced to the marketplace at Alltech’s 1992 international feed industry symposium. The past 14 years have seen numerous successful trials and in the past 12 months meta-analysis summaries of the data in studies with weanling pigs, broilers and turkeys have been published (Pettigrew 2003; Hooge, 2003a; Hooge, 2003b). One researcher working on modeling approaches to use in evaluating Bio-Mos® confirmed that he has found nearly 300 publications in this area (G. Rosen, personal communication). The resounding conclusion: the product is stable in feed, acceptable to the consumer, and works as well if not better than AGPs in comparison studies and on commercial farms. Analysis of the broiler data show a 2% improvement in FCR, a 2% improvement in weight gain, and a 20% decrease in mortality. It clearly has lived up to its motto: Bio-Mos®: Performs. Promise. Its mode of action targets intestinal health and immune modulation. The mannan fraction of Bio-Mos® carbohydrates provides a ‘decoy’ to which pathogens adhere, thereby avoiding intestinal epithelial colonization, which in turn leads to healthier villi and more absorption of nutrients. Immune responses are modulated (as opposed to stimulated directly), leaving the animal more prepared when exposed to pathogens.

The message with Bio-Mos® is that animal health, beginning with gut health, is the key to success.

PERCEPTION: AGRICULTURE POLLUTES

The latest restriction to be placed on animal production in an increasing number of markets is the mandated reduction in dietary copper and zinc in order to prevent accumulation in soil profiles (Figure 2). Supranutritional levels have traditionally been included in monogastric diets, especially those fed pigs, to reduce enteric disorders. Mandated reductions, however, allow only nutritional minimums at a time when many are questioning whether such levels are adequate to meet demands of modern genetic lines.

Can animal health and productivity survive with reductions of critical trace minerals to 20-30% of
their current levels? The answer is yes, providing that dietary trace minerals are supplied in forms best suited to the intestinal environment and absorptive mechanisms. Before reaching the site of absorption (the enterocyte membrane) ingested minerals first encounter an unstirred water layer and then a mucus layer with an intense negative charge (Figure 3). This means that though the enterocyte membrane is very thin, the mineral must first traverse two layers, which are orders of magnitude thicker than the absorptive surface itself. For inorganic metal ions such as Cu, Zn, Mn and Fe, an immediate danger is so-called ‘hydroxy-polymerization’ whereby the increasing pH in the small intestine, and particularly in the unstirred water layer, causes them to form large insoluble metal hydroxides that cannot be absorbed.

The negatively charged mucus layer presents another barrier against the passage of inorganic metal ions and evolved as a protective mechanism against toxic elements such as aluminum (Al^{3+}). Because of the intense negatively charged nature of this layer, the strength of metal cation binding can be described as follows; M^{3+} > M^{2+} > M^{+} (where M represents a metal ion). Essentially, toxic elements such as Al^{3+} are bound so tightly that they rarely manage to traverse this layer and are sloughed off as the layer is replaced. As the charge on the metal ion decreases, inorganic metal ions (which have avoided hydroxy polymerization) may traverse the layer, but at relatively slow rates. This is basically why ferric iron (Fe^{3+}) must first be reduced to ferrous iron (Fe^{2+}) before it can be absorbed.

Feeding essential trace metals in the form of Bioplexes circumvents these problems by a) completely avoiding the risk of hydroxy polymerization reactions, and b) speeding the rate of passage of the metal ion across the negatively charged mucus layer by presenting it in a reduced charge or electrically neutral form (Figure 4).

When dietary trace minerals are in this form, the nutritional minimums mandated by environmental laws are able to meet the needs of modern, highly prolific genetic lines. In studies comparing Bioplex™ and inorganic zinc for grow-finish pigs Fremaut (2003) demonstrated that Bioplex™ Zn supplied at 30% of the inorganic Zn level resulted in improved daily gain while the environmental goal of reduced excretion was accomplished.

![Figure 3. Barriers to absorption of highly charged inorganic cations: formation of unabsorbable hydroxy polymers in the unstirred water layer and adherence to the negatively-charged mucus layer.](image-url)

![Figure 4. General structure of a Bioplex trace mineral.](image-url)
PERCEPTION: RENDERED ANIMAL BY-PRODUCTS IN FEED – DO WE HAVE AN ALTERNATIVE?

While the antibiotic issue can be put aside safely with a tried and proven replacement, and bioplexing allows lower trace mineral levels, this cannot be said of animal by-products. In the US alone, 35 million cattle are processed every year. What could we possibly do with the waste protein and fat? Europe has grappled with this problem, but if the US reduces its use of animal by-products, the impact on protein prices will be enormous.

New plant and yeast protein sources: The ‘Biorefinery’

The nutritional, cost and environmental problems of not recycling animal by-products has no simple solution, but perhaps the ‘biorefinery concept’, at work in the rapidly expanding fuel ethanol industry, can provide a useful alternative protein source. Fuel ethanol is produced in either the grain dry milling or wet-milling process, using a variety of starch and sugar substrates across the globe. Grain dry mills currently produce ethanol, distiller’s dried grains with solubles (DDGS) and CO₂. Removal of the starch for fermentation to ethanol leaves the protein, minerals and fat concentrated in co-products currently used in animal feeding, primarily ruminants but increasingly in monogastrics. With ~30% CP, energy equal to the original grain owing to concentration of fat and ~0.7% phosphorus (90% of which is available), these co-products have much to offer the food animal industry in terms of addressing a protein shortage, but can we improve them further? The ‘biorefinery’ approach to processing starch/sugar sources says yes!

Dry mill ethanol plants using corn produce about 30 kgs of DDGS for each 100 kgs of corn ground. A ‘biorefinery’, in contrast to an ‘ethanol plant’ integrates process streams such that a number of products are produced, with ethanol being only one of potentially many. Options for further processing of spent grains and solubles include secondary fermentations to increase protein content, boost lysine content as much as 3-fold and decrease the indigestible fiber. Enzymatic hydrolysis of DDG and/or solubles is another approach to add flexibility. Ethanol producers seeking to expand the market for distillery co-products have begun integrating processes that ‘re-ferment’ a portion of the solubles and spent grains to provide specialty ruminant products such as VA101 (Figure 5). Such directions go well beyond simply upgrading a ‘by-product’.

Alltech is essentially a yeast biorefinery (Figure 6), constantly examining ways of utilizing yeast or their components. In applying the biorefinery concept to our use of yeast; so another high quality protein for animal feeds arises. In addition to a wide range of specialty yeast applications from animal feeds to ethanol, processes that utilize cell wall fractions in production of Bio-Mos® and Mycosorb® yield a form of yeast extract, which includes the highly nutritious cell contents. It is this extract that is processed into NuPro™, a yeast protein high in nucleotides with application in a broad spectrum of specialty diets, particularly those for neonates of all species.

The lesson of NuPro™, however, is not just that possible new proteins are available in increasing quantities; the message is that innovative research and process results in innovative products if we think outside the box and develop new technologies.

PERCEPTION: ‘MYCOTOXINS ARE NOT IN ANIMAL FEEDS SO WE ARE DOING NOTHING ABOUT THEM’

Like other food safety issues, mycotoxins are a subject that consumers can be expected to be increasingly familiar with in upcoming years. Regulators are extending guidelines to include mycotoxins other than aflatoxin as science provides more and more information about these toxins.

The increasing scientific information about toxin chemistry and function provides us an advantage, however, since it gives us an ability to solve the problem. Knowledge about mycotoxin structural chemistry provides clues useful in building adsorbents. The 3-dimensional structure of yeast cell wall glucan, the starting material for Mycosorb®, can be manipulated to optimize toxin-cell wall interaction making a ‘glucan web’ to prevent toxins from affecting the animal or its products (Figure 7).

Figure 7. Three dimensional structure of yeast cell glucan.
Figure 5. From distillery to biorefinery.

Figure 6. A yeast biorefinery.
Comparing commercially available adsorbents has become a necessity for feed manufacturers. Table 5 contains a 7-point guideline for evaluating such products.

Table 5. 7-point comparison for mycotoxin adsorbents.

1. Can the product adsorb a wide range of toxins?
2. Is inclusion rate sufficiently low (ie. 0.5-2.0 kg/t)?
3. Is the adsorbent stable in the pH range of the GIT?
4. Is adsorption capacity high (will it not be overwhelmed at high toxin concentrations)?
5. Is adsorption affinity high (is it effective at low toxin concentrations since mycotoxins are often toxic at low concentrations)?
6. Is adsorption sufficiently rapid?
7. Are there in vivo data that show protection of production animals against toxins?

Mycosorb®, with its low inclusion rate and structure adapted to adsorb a range of mycotoxins including aflatoxin, zearalenone, T-2 and DON, is rapidly becoming the adsorbent of choice global. Protected by three patents, Mycosorb® has unlimited potential as we learn more about its structure and how modifications can increase adsorption of both known and newly-identified mycotoxins. Again, the appliance of science to solve a practical problem. The fact is that the technology is available to prevent mycotoxins from having an impact at even the animal level, which means that toxins from this source need not threaten food safety in either perception or reality.

Key Success Factor No. 3:
Designing policies for the future: transparency and innovation

Even if we adequately address price competitiveness and consumer perception, in order to be sustainable we need policies that maintain transparency and spur innovation in both products and business strategies.

A key step in defining those policies is deciding where we stand with regard to change: are we going to be proactive or reactive? Is it something that is going to happen to us or will it be something we make happen?

CHANGE IS CONSTANT

Change is inevitable in the dynamic animal feed market, and failure to change has been the death knell of many enterprises. Once we accept that change is a constant, our main decisions revolve around how to deal with change. We can either embrace change and move forward, or we can ignore it until change is forced upon us.

Two large companies whose strategies for change are apparent to us all are McDonald’s® and Starbucks®. The fast-food industry ‘re-invented’ eating out; and for years seemed immune to recession. Recently they have begun to feel the pinch as they have watched consumers ‘re-invent’ what is ‘good’ about food. As a result McDonald’s® stopped buying beef produced using antibiotic growth promoters, they refuse genetically modified potatoes, and in Great Britain have begun to provide organic milk. US McDonald’s® franchises offer ‘Atkins-friendly’ meals for the growing number of carbohydrate-counting customers. Is this a case of McDonald’s® being proactive about changing menus, or are they being reactive when forced to change?

Starbucks® ‘re-invented’ stopping for a cup of coffee with huge success, but now they have begun to add ‘Fair Trade’ and environmentally friendly products. With Conservation International they have collaborated on a project to encourage sustainable agricultural practices and biodiversity through the production of shade-grown coffee, which follows the Institution of Coffee Purchasing’s guidelines. Is Starbucks listening to the consumer or is Starbucks® being proactive?

The changes in McDonald’s® and Starbucks® are examples of transparency and proactive efforts to offer products modern customers are interested in buying. They want customers to know of their commitments to food quality, safety and sustainable agricultural practices. Is our industry just as proactive? Have we lost sight of what Dan Glickman (former US Secretary of Agriculture) advised at the Alltech International Feed Industry Symposium in 2000 – “Tell us what you want and we will grow it”?

Success Factor 3
Designing a sustainable policy for the future

Meet change
Listen and act

head-on
Take on new technologies before competitors do
Make transparency standard

Differentiation
Avoid the ‘sameness’ trap
Choose exceptional, passionate people

Innovation
Creative products
Creative R&D strategies
In order to be sustainable, companies must avoid the ‘sameness trap’ described in Funky Business by Nordström and Ridderstråle (2000). They describe an oversupplied world of similar companies, employing similar people, with similar educational backgrounds, coming up with similar ideas, producing similar things, with similar prices and similar quality. Does this sound like our industry? It does, and it underscores our need to differentiate. We need to create new solutions to problems and in doing so create profit and success for ourselves and our partners. We can make our companies, and hence our industry, different and make them stand out in the industry.

The people factor: exceptional people help companies differentiate

‘Our people make the difference’ must be more than a well-meaning cliché. Many business commentators believe that we are entering an era where the ‘war for talent’ is the most important battle that will be fought. When land was the important asset, countries battled for it, now that talent is the important asset for business success, companies will battle for talent. Paul Allaire, former CEO at Xerox®, calls it “the brawl with no rules”.

What kinds of talents are we looking for? It is one of the fundamental roles of the leader that he/she develop the talent around him/her. Inside rapidly-growing Alltech the need to ensure that the next generation of leaders is in place has been acute. We have key questions to ask potential employees – the most important of which is: What are you passionate about? There is no right or wrong answer, it is simply important to find people with the energy and drive for accomplishment. We have successfully made the transition from a small local player into a medium-sized global enterprise. The next challenge for our people and for our industry involves becoming the industry standard bearer. Part of our future success will be due to recruiting talented and diverse individuals from across the world, including a greater proportion of women, a group whose skills and management styles have been underutilized in agribusiness.

Foster innovation

In the ‘over-supplied world’ described by Nordström and Ridderstråle, ideas are what separate successful companies (and individuals) from failures. Another important element of the future viability of our industry will be our ability to give consumers not only what they want, but more importantly what they did not realize they wanted. The new competition will take place not only in terms of market share, but more importantly in newly created markets. Innovation, while a term vastly overused, is a competency that Alltech and all companies need to excel at in order to prosper.

I was once asked how it could be possible to take a commodity item like milk and make it unique – a value-added product. Is it simple? No. Is it possible? Absolutely. We created a slogan: ‘A milk for all ages’. For the young, a lactoferrin-rich milk for the lactose-intolerant. For teenagers, perhaps higher calcium levels for growing bones; while low fat, high omega-3 and high cholesterol-blocking statin might form part of ‘milk for middle ages’. For all ages, enrichment with selenium through Sel-Plex® in the cow’s diet to fight against cancer. A Korean company went further and changed the name from milk to SELK to emphasize selenium enrichment.
Tatua, with only 30,000 cows is still the world’s most profitable, largely due to the value-added dairy products it offers such as lactoferrin for infant formulas and lactoperoxidase as a natural sterilant.

Alltech’s Bioscience Centers, where scientists complete research toward MSc and PhD degrees while working with Alltech’s research group, are at the hub of our innovation. We support these scientists’ efforts and encourage creative thinking. Over time, 9 PhD and 42 MSc students contributed to the research on Yea-Sacc<sup>1026®</sup>, now the world’s No. 1 natural rumen modifier, which is the reason we understand its mode of action so well. A good example of the impact of this work is the recently obtained EU approval for Yea-Sacc<sup>1026®</sup> in horses. In the US alone we support work being conducted by 36 doctoral candidates and have 135 ongoing projects in Europe.

**Summary**

Re-imagining the feed industry means re-imagining our companies: our goals and what we stand for, our people and the corporate environment we create. We must ask and answer carefully the questions ‘Are we fostering the creativity we need to carry the company into the future? Do our products and research directions address industry needs for price competitiveness and consumer perception? Are the policies sustainable?’

At Alltech, we recognize the importance of ongoing discussion of these questions in building a dynamic corporate culture. It has allowed us to focus on core competencies to develop a ‘Big 6’ list of product directions while giving us the freedom to find ways to expand to a ‘Big 8’ or ‘Big 10’.

Another result of this corporate dynamic is the growing role of the Bioscience Centers as hubs of innovation, both in scientific exploration and in the structure of modern corporate agricultural research—our relationships with other research groups at universities and institutes.

The process is exciting; and it is providing products that have increasing importance across the world in the areas of animal health, performance and reproductive efficiency, and consumer perception of food animal products. Clearly decisions we make surrounding Price, Perception and Policy define ultimately where each of our companies will be in 10, 20 or 30 years’ time.

**References**


Future of the feed/food industry: re-inventing animal feed

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Challenges and opportunities facing the international feed/food industry have never been greater than those faced by these industries today. It has become apparent that anything less than a very proactive approach to addressing current challenges may not be sufficient. Current international events are leading feed companies to attempt to move from a traditional role as a ‘feed company’ to becoming an integral part of the ‘Food Supply Chain’. This is driven essentially by the fact that although one cannot ensure that food company products eaten by consumers are safe, as a prominent player in the early part of the food chain, it is essential that the feed industry ensure that the feedstuff supply chain is not the problem.

This new reality has led feed companies to initiation of the concepts of oversight, control, and overall security of their component of the food chain. Feed companies are, out of necessity, now becoming ‘Food Safety Guardians’. The commitment by the feed company to provide customers with products of the highest quality has been deemed vital to the success of the business. Therefore, feed companies must become early leaders in assuring that all products are Hazard Analysis and Critical Control Point (HACCP) certified. In addition, it is essential that feed companies become registered in the ISO 9001 quality standard or adopt a similar standard. HACCP is a systematic approach to identifying and preventing contamination of food and food products during the manufacturing process. ISO certification is an internationally recognized quality management system that emphasizes integrity throughout the manufacturing process, using standardized and verifiable procedures in all aspects of operations from product design through manufacturing and distribution. The same level of concern has progressed through the livestock and poultry production chain to include producers, integrators, processors, and retailers.

Three key issues asked of the industry today are 1) what are animals consuming, 2) how are animals being cared for, and 3) has the animal been sick? The traditional protein business chain from vegetable to animal protein has changed dramatically. Traditionally, this has been a production-based model from the farmer to the consumer with little oversight. The reality today is that the consumer is providing oversight to the retailer who then places constraints on the production chain to conform to specific standards of production. Overall confidence in food safety was down in the early 1990s, tended to rise and peaked in the mid 1990s and has declined since (Figure 1).

Another issue being addressed by the multinational feed companies is globalization. Given all the issues being addressed internationally, specific feeds need to be modified to fit specific country labeling. This presents tremendous difficulties in developing branded products with international acceptance. Solutions to these issues are exacerbated by the multitude of regulatory hurdles that must be overcome. These include differences in the use of antibiotics, animal proteins, genetically modified materials, and feed additives. Companies that supply inputs are required to think as globally as processors and retailers.

These issues were in the implementation stage prior to the discovery of BSE in North America. All components of the feed/food industries are being affected by this discovery. This is leading to the rapid development and implementation of a national identification program in the US. The ID program will likely bring rapid adoption of Radio Frequency Identification Devices (RFID) in production and processing sectors of the food supply chain and will match the drive of Wal-Mart’s RFID technology in
the retail sector. There will be public pressure for the US government to fund the development and implementation of the national animal ID program, perceived as essential for traceability in a comprehensive food safety program. The need for traceability has been the greatest cost impediment to the adoption and implementation of country-of-origin labeling (COOL). The Manitoba Pork Council has initiated a pilot swine traceability study as part of a national effort to identify the most cost efficient method of tracing swine movement in Canada.

The lack of qualified workers has been and continues to be a major constraint for those associated with the integrated livestock and poultry industries. A survey of students enrolled in swine production in the top swine-producing US states was conducted by Duane Reese (Table 1). This study indicates that interest among students had declined in 9 out of the top 10 swine-producing states over the last five years, with an average decline of 28% in the number of students enrolled. Many other states reported that a lack of interest among students is resulting in swine production not being offered or offered on alternate years. Similarly, poultry production is only offered by a limited number of universities and interest in a poultry production major is low. It is worth noting that this lack of interest comes at a time when world meat demands are expected to increase as developing countries have more disposable income, with a projected increase of 50% by the year 2025 (Elam, 2004). At the same time, the acreage of row crops is projected to decrease over that same time period. Thus, it is imperative to improve production efficiency in both the livestock and crop sectors to be able to meet the rising demands. The other huge area is the need to revamp production agriculture so that all components can be brought back to consistent profitability.

Table 1. Students enrolled in swine production in top 10 swine producing states.

<table>
<thead>
<tr>
<th>State</th>
<th>Fall 1998/</th>
<th>Fall 2002/</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spring 1999</td>
<td>Spring 2003</td>
<td></td>
</tr>
<tr>
<td>Iowa</td>
<td>97</td>
<td>75</td>
<td>↓22</td>
</tr>
<tr>
<td>North Carolina</td>
<td>64</td>
<td>30</td>
<td>↓53</td>
</tr>
<tr>
<td>Minnesota</td>
<td>12</td>
<td>0</td>
<td>↓100</td>
</tr>
<tr>
<td>Illinois</td>
<td>15</td>
<td>14</td>
<td>↓6</td>
</tr>
<tr>
<td>Indiana</td>
<td>30</td>
<td>18</td>
<td>↓40</td>
</tr>
<tr>
<td>Nebraska</td>
<td>11</td>
<td>6</td>
<td>↓45</td>
</tr>
<tr>
<td>Missouri</td>
<td>25</td>
<td>22</td>
<td>↓12</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>37</td>
<td>30</td>
<td>↓15</td>
</tr>
<tr>
<td>Kansas</td>
<td>47</td>
<td>48</td>
<td>↑2</td>
</tr>
<tr>
<td>Ohio</td>
<td>14</td>
<td>10</td>
<td>↓28</td>
</tr>
<tr>
<td>Total</td>
<td>352</td>
<td>253</td>
<td>↓28</td>
</tr>
</tbody>
</table>

*Reese, University of Nebraska, personal communication

Evaluation for technology development by the livestock industry

The swine and poultry industries have made tremendous progress through the years in terms of genetics, nutrition, husbandry and health. Advances in production and management have provided the
marketplace with a high volume, low cost animal protein. Historically the livestock industries have been mostly concerned about commodity production that works best in a least cost, most efficient production model. There are two schools of thought regarding technology evaluation for large systems. The typical approach that has served the integrated livestock industry well in the past is the cost analysis system that is used by Agrimetrics and Agristats. The technique used is a comparison-based cost analysis that compares the cost-of-gain of one company with the cost-of-gain of other comparable enterprises in a manner that keeps the actual companies involved in the analysis confidential. These tools have been largely used to drive systems to ‘least cost’ production with little regard to optimal cost and maximal profit. Some have argued that this system may be overly simplistic and does not effectively address the concept of value returned/cost of input. For example, the most value out of a feeding program may result in a higher cost of gain if the increased cost results in improved performance. The other system is to look at returns over feed costs or a return over input cost. Data analysis services are available that may more effectively address where improvements may be made using a more broad-based cost/benefit analysis. The approach taken by MetaFarms Inc. is to conduct an analysis of ‘Process Enablers’, which affect a number of specific parameters monitored in an enterprise. This leads to a continuing evaluation of the impact that specific processes have on parameters being measured rather than a single focus on cost of gain. One example of this type of analysis is the effect of ractopamine use in a swine production enterprise on performance. Ractopamine added 10 lbs of weight per pig sold, which improved the bottom line by $1.50 to $2.00/pig. Although cost of gain is improved in pigs fed ractopamine, the improvement is minimal compared to the value of improved gain and lean yield; but ractopamine may not be considered unless one analyzes technologies outside a simple cost of gain model. That leaves us questioning how we should evaluate technology. Perhaps the effect a technology has on both the cost side of the equation as well as the revenue side should be evaluated. It is essential that models are developed that evaluate profitability, not just cost of production.

It is also interesting to note that once all the factors with huge effects on performance and efficiency have been implemented, this leaves the livestock industries with the unenviable task of attempting to determine impacts of products and(or) systems that have a much smaller effect on profitability. A 3-4% gain in feed efficiency is almost too small to measure, but the economic impact on profitability in the integrated industry is huge.

Although the challenges are great, much progress is being made in providing alternatives that could benefit the feed/food industries tremendously. The fact that growth-promoting levels of antibiotics are no longer permitted in Europe and the possibility of restrictions being imposed elsewhere has led to a plethora of studies investigating replacements. These studies offer the potential of a better understanding of the relationship between the microbiota in the environment and improved livestock performance as well as alternative strategies to control the threat of specific microorganisms. This may result in improved performance over that observed with growth-promoting levels of antibiotics. Similarly, studies to replace specific animal proteins may lead to a better understanding of factors associated with reduced performance with plant proteins in neonatal animals.

Relationship between the gut microbiota and performance in swine

The gastrointestinal tract of the pig harbors a metabolically active microbiota that stimulates the normal maturation of host tissues and provides key defense functions (Gaskins, 2001). Several recent examples of improved post-weaning performance in the young pig suggest that much of the improvement observed in nutritional studies may be through an impact on the intestinal microbiota. A good argument can be made that the improved performance observed in the young pig as a result of feeding plasma protein, complex diets, antibiotics or acidifiers might be an indirect effect of altering the gut microbiota. Similarly, the positive effects of popular management strategies such as segregated early weaning (SEW) may be mediated through reductions in exposure to pathogens.

Segregated early weaning reduces the incidence of a number of pathogens, thus reducing immunological stress, which results in improved growth and higher efficiency of feed utilization (reviewed by Maxwell, 1999). This strategy has been successful in reducing the number of pathogens, but has not been successful in eliminating all pathogens. The premise is that pigs are removed from the sow while their immunity, as a consequence of maternal antibodies, is still high. This maternally derived passive immunity will
prevent vertical transfer of indigenous pathogens. Pigs reared in isolation have been shown to have reduced immunological stress (Johnson, 1997) resulting in improved growth and efficiency of feed utilization. This is consistent with observations in our research at the University of Arkansas to determine if differences in immune stimulation can explain performance differences in conventional vs off-site reared pigs. A total 432 weanling barrows (19 ± 2 day of age) were obtained from a local commercial company from a single source. One-half the barrows from litters were selected for the off-site nursery study (6 pigs/pen) with the remaining pigs staying in the conventional nursery facilities (approximately 18 pigs/pen). Pigs were weighed and serum samples obtained via venipuncture on days 0, 14, and 34 post-weaning from a total of 72 pre-selected pigs. The pigs were placed in the conventional facilities (a minimum of 1 pig/litter was sampled) and an off-site nursery (a minimum of two pigs in each of 36 pens was sampled). Serum α₁-acid glycoprotein concentrations were determined by a single radial immunodiffusion method using a commercial kit (porcine α₁-acid glycoprotein plate, Development Technologies International, Inc., Frederick, MD). Pigs reared in the off-site nursery were 0.89 kg heavier (P<0.01) at day 14 post-weaning and 2.40 kg heavier (P<0.01) at 34 days post-weaning. In addition, serum α₁-acid glycoprotein concentration was elevated (P<0.01) in pigs reared in the conventional nursery. This suggests that reduced performance in a conventional nursery may be associated with the immunological stress associated with production under these conditions.

The swine industry is implementing early weaning for efficient and economical pig production (Wilson, 1995; Patience et al., 1997). The obvious consequence of weaning is the abrupt change in diet from sow’s milk to solid feed and a change in the environment. There is reduced feed intake during the first week and associated adverse changes in the animal’s gut anatomy and physiology such as villus atrophy, deeper crypts, and infiltration of the villus tip by immature enterocytes (Spring, 1999). Villous atrophy means that there is less absorptive area available for nutrient uptake and deeper crypts represent a large tissue turnover (Spring, 1999). The intestinal microflora can be adversely affected during weaning, resulting in higher numbers of potentially pathogenic acid-intolerant coliforms and a decline of favorable lactobacilli (Bolduan, 1999). In addition, since the piglets are young, their immune systems might not be totally equipped to deal with such pathogenic challenges.

Recently there has been a concern about the use of antibiotics in animal production in part due to antimicrobial resistant bacteria. Over the past two decades, probiotics (direct-fed microbials), which include Lactobacillus cultures, have been used as an alternative to antibiotics in animal production (Jin et al., 1998). Lactobacilli are normal inhabitants of the gastrointestinal tract of pigs. Their beneficial role in the intestinal tract has been attributed to their ability to survive the digestive process, attach to the epithelial lining of the intestinal tract, produce lactic acid and other microbial compounds and prevent colonization by pathogens via competitive exclusion (Savage, 1987). To investigate weaning-induced changes within the enteric system, two experiments were conducted to determine the effect of milk supplementation with Lactobacillus brevis (1E-1) on pre- and post-weaning pig performance, and intestinal microflora. The 1E-1 isolate was sampled from the intestinal tracts of 10 healthy pigs and five pigs with scours, and it was reported that healthy pigs had higher levels of lactobacilli, with the majority of isolates identified as Lactobacillus brevis (Parrott et al., 1994). In each experiment, litters were allotted to two treatments at farrowing: either a control milk supplement, or the control containing 1E-1 via an in-line system using a cup dispenser for each litter. Coliforms and E. coli were enumerated from esophageal, duodenal, jejunal, and ileal regions of the enteric tracts in Experiment 1. Pigs receiving 1E-1 had lower (P<0.05) jejunal E. coli populations pre-weaning and post-weaning compared to pigs provided only milk supplement (Table 2). Ileal E. coli populations were lower (P<0.02) during the post-weaning period for pigs receiving 1E-1 compared to pigs provided milk replacer without 1E-1. The administration of 1E-1 prior to weaning may deter the detrimental alterations in the microbial population that occur at weaning as has been observed in pigs fed zinc oxide (Katouli et al., 1999). During the pre-weaning period (birth to weaning), administration of 1E-1 tended to increase weight gain (Figure 2, P<0.06). During the first five days post-weaning, pigs fed 1E-1 prior to weaning had greater ADG (Table 3, 277 vs 194 g/d; P<0.05) compared to pigs provided only milk replacer, and overall ADG was improved in pigs fed milk replacer with 1E-1. Pigs previously fed milk replacer with 1E-1 were 1.93 kg heavier at the completion of the 28-day study when compared to those receiving the milk replacer alone (Figure 3).
Table 2. Pre- and post-weaning mean $E. coli$ populations in the jejunum and ileum of pigs (CFU/g log10).

<table>
<thead>
<tr>
<th></th>
<th>Pre-wean</th>
<th>Post-wean</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1E-1</td>
</tr>
<tr>
<td>Jejunum</td>
<td>5.53a</td>
<td>3.42b</td>
</tr>
<tr>
<td>Ileum</td>
<td>5.91</td>
<td>4.71</td>
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</table>

$^{a,b}$Pre- and post-wean means within a row with different letters differ significantly ($P<0.05$).

Table 3. Effect of $Lactobacillus brevis$ (1E-1) during lactation on subsequent nursery pig performance (Experiment 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Milk</th>
<th>Milk + L. brevis (1E-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1 (day 0-5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, g</td>
<td>194$^a$</td>
<td>277$^a$</td>
</tr>
<tr>
<td>ADFI, g</td>
<td>91</td>
<td>109</td>
</tr>
<tr>
<td>Phase 1 (day 0-14)</td>
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<td></td>
</tr>
<tr>
<td>ADG, g</td>
<td>211</td>
<td>258</td>
</tr>
<tr>
<td>ADFI, g</td>
<td>211</td>
<td>250</td>
</tr>
<tr>
<td>Overall (day 0-28)</td>
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<td></td>
</tr>
<tr>
<td>ADG, g</td>
<td>355</td>
<td>388</td>
</tr>
<tr>
<td>ADFI, g</td>
<td>435</td>
<td>461</td>
</tr>
<tr>
<td>Gain:feed</td>
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<td>0.85</td>
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<tr>
<td>Weight, kg</td>
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<td></td>
</tr>
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<td>Initial</td>
<td>5.50$^c$</td>
<td>6.31$^a$</td>
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<tr>
<td>Phase 1</td>
<td>8.26</td>
<td>9.88</td>
</tr>
<tr>
<td>Phase 2</td>
<td>15.19</td>
<td>17.12</td>
</tr>
</tbody>
</table>

$^a$Letters that differ within the same column are different ($P<0.02$).

A second study to confirm these results has recently been completed. Beginning at farrowing, pigs were provided milk supplementation either with or without the addition of $Lactobacillus brevis$ (1E-1) via an in-line system using a cup dispenser for each litter. These treatments were continued during the nursery period, in which pigs that were administered 1E-1 via milk supplementation continued to receive 1E-1 through the watering system. Pigs supplemented with 1E-1 had greater ADG ($P<0.05$) during Phase 2 and in the overall nursery period (day 0 to 38), greater ADFI ($P<0.05$) during Phase 3 and the overall nursery period, and tended to have increased gain:feed ($P<0.10$) during Phase 3 (Table 4). In this study, 1E-1 supplementation resulted in a 1.58 kg improvement ($P<0.01$) in body weight at the end of the six-week nursery period compared to pigs not receiving 1E-1 (Figure 4). These data indicate that 1E-1 supplementation pre-weaning improves nursery performance and may provide a healthier intestinal environment.

Studies that I have summarized involve classical culture techniques. We are adapting procedures that allow quantitative determination of all the primary microbiological species in the gut microbiota using molecular techniques (Figure 5). The development of molecular-based methods that by-pass the need for culturing bacteria has led to a renaissance in microbial ecology studies. Molecular methods that utilize the Polymerase Chain Reaction (PCR) exponentially amplify copies of bacterial DNA to literally produce a billion copies of the original template without growing the bacteria. These methods are revolutionizing our understanding of the complex interactions between host and gut microbiota.
methodologies have created the first opportunity for ecologists to gain a true representation of the microbial world as it would exist in natural environments. To examine the bacterial ecology of known and unknown bacteria, the most often used method is to PCR amplify the 16S rRNA/DNA genes from a mixed bacterial community. These highly conserved genes encode a portion of the bacterial ribosome found in every known bacterium. This amplified mix of gene sequences can be separated, sequenced and compared to the 16S rRNA/DNA database presently containing taxonomic data of over 85,000 bacterial sequences.

These studies suggest that the impact of gut microbes on performance in young pigs is greater than once thought. Other researchers have documented enhanced performance in growing/finishing pigs associated with improved health and management. Pigs with minimal disease due to SEW, which were fed a series of non-limiting diets and reared in pens of three pigs (2.23 m²/pig), achieved 104 kg at 136 days of age and 120 kg at 151 days of age (Schinckel et al., 1995). Pigs raised on the original commercial farm, conventionally weaned with all-in, all-out production, required 184 days to attain 104 kg live weight. This dramatic effect of health is likely mediated by inflammatory cytokines or other systemic inflammatory effects in response to bacterial toxins. The overall effect is to down regulate muscle synthesis and growth. Schinckel et al. (2003) presented a review of current research on muscle endocrine, and immune regulation of growth.

**Bio-Mos®**

Although prohibited in many European countries, the addition of antibiotic growth promoters to swine diets remains a common practice in the US, particularly to the diets of newly weaned pigs. However, there has been increasing pressure on the
livestock industry to decrease or discontinue these additions because of the potential development of antibiotic resistance. The need for alternative methods to improve growth and efficiency of livestock production and to modulate the animal’s natural ability to fight disease has prompted the scientific investigation of several feed additives and their ability to positively alter immune function (Berg, 1998; Turner et al., 2001).

Supplementation of swine diets with mannan oligosaccharides derived from the yeast cell wall of *Saccharomyces cerevisiae* has the potential to provide an alternative to growth-promoting antibiotics. Mannan-based supplements have the ability to alter

**Figure 4.** Effect of milk replacer supplementation with *Lactobacillus brevis* (1E-1) on final nursery weight (Trial 2-42 days).

**Figure 5.** Procedural outline to quantitatively determine all species in a mixed culture (adapted from Liu et al., 1997).
the microbial population in the intestinal tract. This modification seems to be accomplished by the ability of mannans to attach to mannose-binding proteins on the cell surface of some strains of bacteria, thereby preventing these bacteria from colonizing the intestinal tract by interfering with the binding of carbohydrate residues on epithelial cell surfaces (Spring et al., 2000). Mannans have also been reported to alter immune function in swine (Kim et al., 2000), and this may be an additional mechanism by which mannans improve growth performance.

The effects of Bio-Mos® on pig performance and immunocompetence was evaluated in five nursery pig trials conducted at the University of Arkansas. A total of 412 pigs were included in the evaluation with 82 total observations (38 pens fed the basal diet, 15 pens fed 0.2% Bio-Mos®, and 29 pens fed 0.3% Bio-Mos®). In four of the five trials, Phase 1, Phase 2, and Phase 3 were defined as day 0 to 10 after weaning, day 10 to 24 after weaning, and day 24 to 38 after weaning, respectively. The fifth trial consisted of a 14-day Phase 1, and a 7-day Phase 2. During Phase 1, Bio-Mos® supplementation improved (P<0.02) feed efficiency compared to pigs fed the basal diet, whereas improvement in ADG approached significance (Table 5, P=0.11). Pigs supplemented with 0.3% Bio-Mos® had improved feed efficiency for the overall Phase 1 and 2 periods (P<0.03) when compared to those fed the control diet. During the first week of Phase 3, ADG (P<0.05) and feed efficiency (P<0.05) were improved in pigs fed Bio-Mos® when compared to pigs fed the basal diet. There were no differences (P>0.20) in lymphocyte proliferation between pigs fed Bio-Mos® and those fed the basal diet when data from the five trials were combined. However, evaluation of immune assays conducted in the fifth trial revealed that Bio-Mos®-supplemented pigs had a greater proportion of lymphocytes in the peripheral blood (P<0.03), an increase (P<0.10) in the proportion of macrophages in the jejunal lamina propria, and an increase (P<0.05) in the phagocytic capacity of jejunal lamina propria macrophages compared to pigs fed the basal diet (P<0.05, Table 6). Data compiled from five experiments conducted at the University of Arkansas conclude that Bio-Mos® supplementation improves weight gain and feed efficiency in nursery pigs. Although the function of lymphocytes derived from peripheral blood was not affected in nursery pigs, the results of these studies with Bio-Mos® supplementation, Bio-Mos® did enhance innate immune function in the gastrointestinal system.

| Table 5. Summary of the effect of Bio-Mos® on growth performance in 5 trials. |
|---|---|---|---|
| Bio-Mos® (kg/t) | 0 | 0.2 | 0.3 |
| ADG, g | | | |
| Phase 1c | 147 + 9 | 168 +16 | 166 +10 |
| Phase 2 | 372 + 12 | 369 + 22 | 391 +14 |
| Phase 3d | 461 + 14 | 516 + 23 | 501 +18 |
| Feed:gain | | | |
| Phase 1d | 1.709 + 0.067 a | 1.388 + 0.126 b | 1.493 + 0.080 b |
| Phase 2 | 1.437 + 0.085 | 1.428 + 0.160 | 1.195 + 0.104 |
| Phase 1-2f | 1.379 + 0.025 ab | 1.375 + 0.046 ab | 1.273 + 0.030 b |
| Phase 3d | 1.700 + 0.028 a | 1.586 + 0.046 b | 1.610 + 0.036 ab |
| a,bMeans in a row with no letters in common differ (P<0.05). |
| cContrast: 0 vs 0.2 + 0.3% Bio-Mos®; P = 0.11 |
| dContrast: 0 vs 0.2 + 0.3% Bio-Mos®; P<0.05 |
| eContrast: 0 vs 0.2 + 0.3% Bio-Mos®; P<0.10 |
| fContrast: 0.2% vs. 0.3% Bio-Mos®; P<0.10 |

Our data indicate that Bio-Mos® alters the proportion and function of leukocytes isolated from the peripheral blood as well as the gastrointestinal tract of the weanling pig. One of the questions raised by the results of these studies with Bio-Mos® is; how can a non-digestible feed additive alter immune responses in the gastrointestinal tract and systemically in the pig? One possible mechanism is via the uptake of Bio-Mos® from the lumen of the gastrointestinal tract by M cells of Peyer’s patches. Peyer’s patches are organized lymphoid follicles located along the luminal surface of the small intestine. Dispersed
throughout the epithelial layer of the Peyer’s patch are specialized epithelial cells, termed M (membranous) cells, which function to pinocytose and transport macromolecules from the intestinal lumen into the subepithelial tissue, delivering antigenic molecules to leukocytes within the Peyer’s patch. The extraction of Bio-Mos® from the lumen of the small intestine by the M cells of the Peyer’s patch and its exposure to the immune cells located there, may be the impetus for a cascade of immunomodulatory events that develop and enhance immune function, both locally in the gastrointestinal tract as well as systemically, as cells migrate out of the gastrointestinal tissue into the periphery.

Because mannan oligosaccharides have been documented to alter bacterial populations within the intestinal tract (Spring et al., 2000), another explanation for the alterations in immune function observed in these studies may be from changes elicited in the enteric microbial population by the presence of mannans in the luminal environment of the intestinal tract. The microflora present in the gastrointestinal tract are known to be a factor in the development of the young pig’s immune system, both enterically and systemically (Gaskins, 1997), and the alteration of these microbial populations by Bio-Mos® could have an impact on the progression of immune system development.

**NuPro™ 2000**

Pigs produced in conventional intensively managed swine production systems are routinely weaned at 19 to 21 days of age and as early as 10 to 14 days of age in off-site SEW systems. At this age, pigs are very sensitive to the source of dietary protein. Many dietary proteins produce allergic reactions in which diarrhea, reduced growth and increased mortality can occur (Bimbo and Crowther, 1992). Various protein sources have been tested in early-weaned pig diets in an attempt to overcome these problems and to decrease diet cost. Spray-dried plasma protein is a protein source that has consistently been shown to improve performance of early-weaned pigs when included in Phase 1 (day 0 to 14 post-weaning) diets at the expense of dried skim milk (Hansen et al., 1993; Kats et al., 1994; de Rodas et al., 1995), soybean meal (Fakler et al., 1993; Coffey and Cromwell, 1995; de Rodas et al., 1995), and whey (Hansen et al., 1993). Select grade menhaden fish meal has also been a widely utilized protein source due to a combination of consistent quality and competitive price. Demand for plasma protein is high and supply is limited, therefore plasma is an expensive protein source for nursery diets. Also, regulatory constraints that prohibit the use of plasma protein in many countries may affect the use of bovine plasma in the US. Similarly, increased demand and decreased supply of fish meal has resulted in increased price volatility and relatively high current prices.

Preventing intestinal damage or atrophy immediately post-weaning caused by reduced feed intake and lack of stimulation of the intestinal epithelium by ingested particles has been suggested as important in maintaining growth performance in nursery pigs (Cera et al., 1988; Dunsford et al., 1989). However, there are many other factors, including removal of beneficial factors from sow’s milk, diet form, stress, invasion by microorganisms, or introduction of allergenic compounds in the nursery diet, that may also contribute to intestinal atrophy. Glutamic acid and nucleotides may be important nutrient sources for maintaining gut integrity during the early nursery period.

NuPro™ 2000 is a protein source high in crude protein (51 to 55%) and digestible amino acids that has potential as a possible alternative protein source in nursery diets. NuPro™ is also high in glutamic acid and is an excellent source of nucleotides. Several animal-based specialty feed ingredients have been developed to compete against the animal plasma and fish meal market share. However, NuPro™ is a vegetable-based peptide product which may have greater international market appeal compared to products originating from animal by-products and the high level of nucleotides may be uniquely beneficial to the early-weaned pig.

A study has been completed at the University of Arkansas involving a total of 216 pigs to evaluate the efficacy of feeding NuPro™ as an alternative to plasma protein in nursery pig diets (9 pens/treatment). Three dietary treatments were fed from day 0 to 7 after weaning (Phase 1) and day 7 to 21 after weaning (Phase 2) and were comprised of 1) a basal diet consisting of a complex nursery diet containing spray-dried plasma protein devoid of NuPro™, 2) the basal diet with 50% of the plasma protein replaced by NuPro™, and 3) the basal diet with 100% of the plasma protein replaced by NuPro™. During Phase 3 (day 21 to 42 after weaning) a common diet was fed to groups previously receiving Treatments 1 and 2. Half of the pigs previously fed Treatment 3 were fed the common diet received by the Treatment
groups 1 and 2; while the other half were fed a diet containing 1.3% NuPro™ during the first week of Phase 3 (day 21 to 28) followed by the common diet for the remainder of the phase (day 28 to 42). During Phases 1 and 2, no significant differences were observed among the four dietary treatments with regard to ADG, ADFI, or G:F (Table 7). During the first week of Phase 3, pigs previously fed the basal diet containing plasma protein and fed NuPro™ at the 50% replacement level had lower (P=0.07) ADFI than pigs previously fed NuPro™ at the 100% replacement level and pigs fed NuPro™ at 1.3% of the diet during the first week of Phase 3. This study indicates that NuPro™ maybe used as an alternative to spray-dried plasma protein in nursery pig diets, and the removal of NuPro™ from the diet does not result in decreased feed intake as is often the case with the removal of plasma protein. This study indicates that NuPro™ may be an effective replacement for plasma protein.

**Organic selenium from yeast**

Organic selenium offers several major opportunities to the feed/food industries. Although inorganic selenium (sodium selenite) has routinely been added to most animal diets, research has shown that about 60% of it is excreted in the urine. Organic selenium (selenium enriched yeast) is an effective source of selenium as it is more effectively retained in muscle, milk, and fetal tissues than inorganic selenium and less is excreted. Accumulation in tissues provides a selenium reserve that can be used under conditions of stress for additional synthesis of selenoproteins essential for counteracting adverse effects of free radicals. Organic selenium is also transferred into the egg more efficiently and into embryonic tissues in mammals via improved placental transfer when compared to sodium selenite. This provides the young animal with higher selenium stores, which can promote improved disease resistance. In addition to the benefits to livestock species, higher tissue levels of organic selenium may offer health benefits to consumers who choose Se-enriched animal products. Although somewhat controversial, increased selenium intake has been associated with reductions in cancer risks in epidemiological studies (Vogt et al., 2003), animal models (Popova, 2002) and chemopreventive studies (Combs et al., 2001; Clark et al., 2000; Clark et al., 2001).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>SE</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>50% NuPro™</td>
<td>100% NuPro™</td>
<td>100% NuPro™</td>
<td></td>
<td></td>
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<tr>
<td>ADG, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Phase 1</td>
<td>47</td>
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<td>10</td>
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<tr>
<td>Phase 2</td>
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<td>429</td>
<td>404</td>
<td>11</td>
<td>0.19</td>
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<td>Phase 1-2</td>
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<td>283</td>
<td>296</td>
<td>281</td>
<td>9</td>
<td>0.58</td>
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<tr>
<td>Phase 3</td>
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<td>590</td>
<td>626</td>
<td>621</td>
<td>12</td>
<td>0.17</td>
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<td>451</td>
<td>437</td>
<td>461</td>
<td>450</td>
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<td>ADFI, g</td>
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<td></td>
<td></td>
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<td>99</td>
<td>116</td>
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<td>465</td>
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<td>354</td>
<td>365</td>
<td>349</td>
<td>11</td>
<td>0.78</td>
</tr>
<tr>
<td>Phase 3</td>
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<td>962</td>
<td>1037</td>
<td>1002</td>
<td>19</td>
<td>0.08</td>
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<td>Phase 1-3</td>
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<td>658</td>
<td>701</td>
<td>675</td>
<td>13</td>
<td>0.18</td>
</tr>
<tr>
<td>Gain:feed</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Phase 1</td>
<td>0.393</td>
<td>0.376</td>
<td>0.266</td>
<td>0.272</td>
<td>0.077</td>
<td>0.53</td>
</tr>
<tr>
<td>Phase 2</td>
<td>0.845</td>
<td>0.850</td>
<td>0.868</td>
<td>0.874</td>
<td>0.012</td>
<td>0.25</td>
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<tr>
<td>Phase 1-2</td>
<td>0.795</td>
<td>0.799</td>
<td>0.816</td>
<td>0.810</td>
<td>0.011</td>
<td>0.54</td>
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<tr>
<td>Phase 3</td>
<td>0.615</td>
<td>0.615</td>
<td>0.603</td>
<td>0.613</td>
<td>0.008</td>
<td>0.71</td>
</tr>
<tr>
<td>Phase 1-3</td>
<td>0.662</td>
<td>0.665</td>
<td>0.658</td>
<td>0.663</td>
<td>0.006</td>
<td>0.87</td>
</tr>
<tr>
<td>Weight, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>6.45</td>
<td>6.45</td>
<td>6.45</td>
<td>6.45</td>
<td>0.005</td>
<td>0.86</td>
</tr>
<tr>
<td>Phase 1</td>
<td>6.78</td>
<td>6.77</td>
<td>6.67</td>
<td>6.69</td>
<td>0.07</td>
<td>0.62</td>
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<tr>
<td>Phase 2</td>
<td>12.37</td>
<td>12.39</td>
<td>12.67</td>
<td>12.35</td>
<td>0.18</td>
<td>0.57</td>
</tr>
<tr>
<td>Phase 3</td>
<td>25.44</td>
<td>24.79</td>
<td>25.81</td>
<td>25.51</td>
<td>0.34</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Group 1: days 1-21, basal diet; days 21-42 common diet.
Group 2: days 1-21, basal with 50% NuPro™ replacement; days 21-42 common.
Group 3: days 1-21, basal with 10% NuPro™ replacement; days 21-42 common.
Group 4: days 1-21, basal with 100% NuPro™ replacement; days 21-28 1.3% NuPro™; days 28-42 common.
and Marshall, 2001). Should additional studies underway prove conclusive, this presents the livestock and poultry industries with an opportunity to provide additional health benefits to the public consuming animal products.

**Environmental impact of concentrated animal feeding units**

Another major problem facing the feed/food industry is the concentration of nutrients in modern livestock and poultry production systems. Northwest Arkansas contains the headwaters for two scenic rivers and is also the location of a major concentration of animal production, primarily poultry. Disposal of the concentrated animal waste, which accumulates in efficient production systems, in a manner that minimizes odor and optimizes nutrient utilization is an increasing problem facing the livestock and poultry industries in our state. Animal waste can be a valuable resource as an alternative source of fertilizer nitrogen (N), phosphorus (P), and potassium (K) in maintaining and restoring soil productivity. In fact, by improving ground cover, runoff volume and erosion may also be reduced. Conversely, application of animal manure at rates greater than a crop can utilize has been shown to result in nitrate (NO₃) movement through the soil into ground water and can result in an excessive rise in soil P levels, leading to increased phosphorus runoff. This can be a problem since phosphorus is normally the limiting nutrient for eutrophication in freshwater systems. Odor and nutrient problems can both be exacerbated by excessive nutrient buildup in lagoons/holding ponds that have not been dewatered in a timely manner.

With the initial population of the new University of Arkansas 2000 head/year finishing facility, a decision was made to demonstrate the use of dietary phytase addition to substantially reduce phosphorus production in swine manure without affecting swine performance or profitability. Facilities were constructed to permit production of two types of manure that was stored in holding ponds. Pigs placed in half of the pens received normal phosphorus diets devoid of phytase and pigs placed in the other half of the facility received diets with reduced phosphorus supplemented with phytase. The holding ponds were managed by emptying the shallow pit under the pigs on each diet on a weekly basis and recharging the pit with effluent from the top of the holding pond. This simulated the management of a pull-plug waste disposal system and allowed the accumulation of the two types of manures for application on watersheds.

Table 8 provides the average total and soluble phosphorus analysed in the holding ponds. The 24.8% reduction in total phosphorus is consistent with the magnitude of reduction observed in phosphorus balance studies with pigs to determine the magnitude of reduction of phosphorus expected by feeding reduced phosphorus diets with added phytase. The magnitude of reduction in soluble phosphorus was only 8.95%, suggesting that a higher percentage of the phosphorus from pigs fed phytase was in the soluble form. This is consistent with other observations that phytase increases soluble phosphorus in manure.

**Table 8. Phosphorus concentration in holding ponds (mg/L).**

<table>
<thead>
<tr>
<th>Item</th>
<th>Normal P</th>
<th>Phytase P</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total P</td>
<td>289.7</td>
<td>217.9</td>
<td>24.8</td>
</tr>
<tr>
<td>Soluble P</td>
<td>138.4</td>
<td>126.0</td>
<td>8.95</td>
</tr>
</tbody>
</table>

N = 6 samples per manure source

The reduced risk of phosphorus runoff from watersheds receiving manure from phytase-treated pig diets in relation to manure from pigs fed normal phosphorus, non-phytase diets was also demonstrated. Concern over water quality near animal production facilities is primarily with regard to transport of excessive amounts of N and(or) P from the animal waste.

A third watershed evaluated the efficacy of aluminum chloride (AlCl₃) addition to swine manure on runoff. Shreve et al. (1995), Moore et al. (1995) and Smith et al. (2001) recommended treatment of manure with aluminum chloride as a means of reducing both P and NH₃ losses. Runoff of nutrients was compared to a watershed that received no manure or fertilizer. The watershed sites were designated:

1. No manure or fertilizer application
2. Phytase manure: Low P diet, high N, but low P loading, lower risk of P runoff.
3. Normal manure: Normal P diet, high N and P loading on pasture, high risk of P runoff.
4. Phytase manure: Low P diet, high N, but low P loading, aluminum chloride added to reduce soluble phosphorus and lower risk of P runoff.

Manure was transported from the respective holding basins and applied to two separate pastures in multiple
applications at rates equivalent to a target of 150 lb N/acre/year. Manure from pigs fed the reduced phosphorus diet with added phytase was also treated with aluminum chloride by adding 0.75% aluminum chloride to the manure prior to application. This was added to a third watershed at the same application rate used in the other watersheds. A fourth watershed had no manure or fertilizer added. A total of three applications were made during the project. A ‘Small In-field Runoff Collector’ system was used to collect runoff water. Samples from each watershed and storm event were composited and analyzed for total Kjeldahl N, total P, soluble P, NH$_3$-N, NO$_3$-N, copper and zinc.

Total runoff data are presented in Table 9. The watershed that received no manure or fertilizer produced the greatest total runoff with 191,344 liters. This might be expected since reduced forage cover may increase runoff. The watershed receiving the normal phosphorus manure was next with 179,028 liters followed by the watershed receiving AlCl$_3$, treated manure from pigs fed phytase (162,418 liters). The watershed receiving untreated manure from pigs fed phytase had the lowest total runoff of 112,826 liters.

Table 9. Total runoff.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(Liters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Watershed 1</td>
<td>No manure</td>
</tr>
<tr>
<td>Watershed 2</td>
<td>Phytase</td>
</tr>
<tr>
<td>Watershed 3</td>
<td>Normal P</td>
</tr>
<tr>
<td>Watershed 4</td>
<td>Phytase + AlCl$_3$</td>
</tr>
</tbody>
</table>

The total nutrients applied to the watersheds in the three applications are presented in Table 10. The application of total N was approximately 150 lbs of total N/acre/year. The addition of aluminum chloride to the swine manure also substantially reduced the soluble phosphorus at the time of manure application, as expected.

Table 10. Nutrients applied to soil from manure by treatment (lb/acre).*  

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soluble P (lb/acre)</th>
<th>Total P (lb/acre)</th>
<th>Total N (lb/acre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfertilized</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Phytase diet</td>
<td>12.7</td>
<td>31.5</td>
<td>230</td>
</tr>
<tr>
<td>Normal diet</td>
<td>10.2</td>
<td>30.4</td>
<td>205</td>
</tr>
<tr>
<td>Phytase + AlCl$_3$</td>
<td>1.6</td>
<td>26.6</td>
<td>240</td>
</tr>
</tbody>
</table>

*Represents three applications over 1.5 years.

The mass of soluble and total P lost from the watershed fertilized with normal manure was greater when compared to the runoff in the unfertilized watershed or watersheds fertilized with phytase manure or phytase manure with AlCl$_3$ (Table 11). This total mass of P loss in the watershed fertilized with normal manure also represented the greatest percentage of applied total P lost among the watersheds (5.4% vs 3.6% and 4.9% for the watersheds treated with phytase manure and phytase with AlCl$_3$, respectively). Application of manure from pigs fed phytase, with or without treatment with AlCl$_3$, reduced the mass of soluble and total P runoff. In fact, the watershed treated with phytase manure produced the lowest total P and percentage of soluble and total P runoff among the watersheds, even lower than that observed in the unfertilized watershed. It should be noted, however, that runoff volumes were variable between watersheds, which had an impact on the total mass of nutrients lost from the runoff events. When comparing the mass of nutrients applied to that which was lost through runoff, it is important to note that the vast majority of nutrients remained within the watershed. In general, more than 90% of the nutrients applied remained in the watershed. The exceptions to this are the percentage of soluble P lost from the watershed receiving the normal P manure (12.7%) and the percentage of soluble P lost from the watershed receiving the phytase manure with AlCl$_3$ (60.6%). A higher percentage of soluble nutrients were lost through runoff, because the soluble fraction is fairly dynamic, and is also more susceptible to runoff losses than the total fraction. There was more soluble P removed from the phytase manure with AlCl$_3$ watershed than was applied from the manure, most likely due to the natural loss of soil P as seen in the unfertilized watershed. The N lost from these watersheds was a very small fraction of what was applied, ranging from 0.6% to 1.2%.

Table 11. Mass of nutrients lost from watersheds and percentage of applied nutrients lost.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soluble P (%</th>
<th>Total P (%</th>
<th>Total N (%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfertilized</td>
<td>0.89</td>
<td>1.26</td>
<td>1.84</td>
</tr>
<tr>
<td>Phytase diet</td>
<td>0.92</td>
<td>7.2</td>
<td>1.12</td>
</tr>
<tr>
<td>Normal diet</td>
<td>1.30</td>
<td>12.7</td>
<td>1.65</td>
</tr>
<tr>
<td>Phytase + AlCl$_3$</td>
<td>0.97</td>
<td>60.6</td>
<td>1.31</td>
</tr>
</tbody>
</table>

Zinc concentrations from runoff were very low (Figure 6). Manure had no apparent impact on metal runoff when applied as a fertilizer resource to the watersheds. Copper concentrations in runoff were also very low, in the ppb range, and were not affected by the addition of manure to the watersheds (Table 12).
Table 12. Concentration of copper lost in runoff by treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cu (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Unfertilized</td>
<td>2.5</td>
</tr>
<tr>
<td>2. Phytase Diet</td>
<td>2.3</td>
</tr>
<tr>
<td>3. Normal Diet</td>
<td>3.6</td>
</tr>
<tr>
<td>4. Phytase + AlCl$_3$</td>
<td>3.5</td>
</tr>
</tbody>
</table>

One of the objectives of this project was to conduct a phosphorus and nitrogen budget for the farm. A total of 8,222 lb of phosphorus was delivered to the farm and an estimated 2,507 lbs (30.5%) was removed in pigs marketed or retained in pigs kept as replacement breeding stock (Table 13). A total of 1,616 lbs (19.6%) was spread on 88 acres for an average application rate of over 12 lbs of total P/acre/yr, which exceeds the P needed for forage production for grazing or hay. The amount of total N delivered to the farm was 40,839 lb (Table 14). An estimated 11,608 lbs (28.60%) was removed in pigs marketed or retained in pigs kept as replacement breeding stock. A total of 3,655 lb (8.94%) was spread on 88 acres for an average maximum application of 41.50 lbs of N/acre. If one obtained the expected ammonia loss from volatilization of 25%, then the actual applied N would be about 31 lb/acre, which is probably below the crop needs for either the bermuda or fescue pastures where manure was applied. The residual N is most likely much less than the calculated residual since ammonia volatilization from the production facility and holding ponds is likely to be substantial.

Table 13. Farm phosphorus balance.

<table>
<thead>
<tr>
<th></th>
<th>lbs</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total P delivered in feed</td>
<td>8,222</td>
<td></td>
</tr>
<tr>
<td>P removed in pigs marketed</td>
<td>2,507</td>
<td>30.5</td>
</tr>
<tr>
<td>P in manure spread</td>
<td>1,616</td>
<td>19.6</td>
</tr>
<tr>
<td>Residual</td>
<td>4,099</td>
<td>49.8</td>
</tr>
</tbody>
</table>

Table 14. Farm nitrogen balance.

<table>
<thead>
<tr>
<th></th>
<th>lbs</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N delivered in feed</td>
<td>40,839</td>
<td></td>
</tr>
<tr>
<td>N removed in pigs marketed</td>
<td>11,680</td>
<td>28.60</td>
</tr>
<tr>
<td>N in manure spread</td>
<td>3,655</td>
<td>8.94</td>
</tr>
<tr>
<td>Residual</td>
<td>25,504</td>
<td>62.45</td>
</tr>
</tbody>
</table>

This study demonstrates that even with judicious management, phosphorus in the soil accumulates with application of swine manure based on plant requirements for N in forage-based systems. Construction of new production facilities should only be considered after development of nutrient management plans ensuring application of nutrients that do not exceed crop needs. Technologies to further reduce phosphorus in manure would reduce the land base needed for concentrated animal production facilities.

In areas where nutrient excesses exist, progress is being made in developing technologies to address the problem and some are even receiving praise for delivering both environmental and economic benefits. Single out in the popular press recently is the manure management system in the Chino Basin in Southern
California, which utilizes a digester to convert dairy manure into fertilizer and methane used for power generation. Smithfield Foods is constructing a $20 million system to convert swine manure into liquid methanol to be used in the production of biodiesel. Success of these systems is critical for maintaining good relationships in communities with large livestock production facilities. Why would every state not want to have a $7 billion poultry industry like Arkansas?

Conclusions

In summary, it appears there will be increased emphasis on food safety that will require dramatic changes in the way national and international feed/food companies operate. This has benefits not only from a food safety standpoint, but as we develop a better understanding of controlling microorganisms in the environment, we may also tremendously improve animal health and performance and should have a number of alternatives to antibiotics. The impact of an animal’s interaction with microorganisms in the environment on gain and efficiency is even greater than once thought. Finally, it is evident that environmental and animal welfare issues will have increasing influence on decision making.

References

Glycomics: putting carbohydrates to work for animal and human health

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Introduction

In October of 2001 bioterrorism came to the United States in the form of letters containing anthrax spores. Imagine a time in the very near future when simply eating a carbohydrate fraction from yeast can protect you from such an attack. It is not as far-fetched as you may think. A recent trial in mice found that mice receiving yeast glucans for 1 week prior to anthrax infection had double the survival rate of unsupplemented animals. As a therapeutic agent, mice receiving yeast glucans had a 90% survival rate compared to 30% survival for control animals (Kournikakis et al., 2003).

In February of 2003, the magazine Technology Reviews identified glycomics as one of the “10 Emerging Technologies That Will Change the World.” Glycomics is defined as the characterization of the sugars and the structure of these sugars that make up a cell. We are all aware of the quest to define the human genome (genomics: the full DNA complement) that was recently completed. Many are aware of proteomics, which is the study of the full set of proteins encoded by a genome, but very few people are aware of glycomics. Putting these sciences in perspective, genomics was child’s play compared to the undertaking of proteomics, which is dwarfed in comparison to glycomics.

New roles for carbohydrates

At one time, it was thought that there were three main roles of carbohydrates in biological systems. The most obvious role of sugar is as an energy source or storage component for energy reserves. The second function is as a structural component such as cellulose or chitin. The third function seemed to be to confound scientists studying proteins and lipids by being associated with these compounds and subsequently needing to be stripped away in order to truly understand the function of the protein. However, it turns out that the glycosylation of these compounds can define their function or serve to stabilize them. A good example of this stabilization is industrial-grade enzymes, where shelf-life and heat stability have been enhanced by glycosylation of the protein.

Unlike amino acids or nucleic acids that have a certain predictability to their structure, there is no simple code for determining the structure of complex sugars. The biological diversity of these compounds can be easily demonstrated by examining the difference between α- and ß-bonded (1-4) glucose units (Figure 1). When these two glucose units are bound in the α configuration, the resulting compound, amylose, is easily degraded by starch-degrading enzymes found in saliva. Conversely, ß-bonded (1-4) glucose represents cellobiose, a compound that is not degraded by any mammalian enzyme system. This exemplifies the difference in biological activity of the same two glucose molecules bound together at the same site with the only difference being the type of bond between them. Compared to DNA or amino acid linkages, which are somewhat linear, imagine the complexity when you consider that a hexose molecule (like glucose) has six binding sites, can branch and the bonds can orient in different ways (as seen in Figure 1). The possibilities for oligo- or polysaccharides can be a bit overwhelming.

Complex carbohydrates have become a prominent research topic with the realization that distinct carbohydrate structures can have very specific biological activities. One need only imagine the
Glycomics: putting carbohydrates to work for animal and human health

The diverse nature of carbohydrate chemistry to understand that the opportunities for novel compounds with unique biological activity are boundless. In fact, the diversity and complexity of these compounds has kept investigators from fully understanding them until recently. Carbohydrates and oligosaccharides are also now being utilized as a nutritional means of enhancing immune function.

Trehalose is a disaccharide of two glucose molecules linked by α(1,1) linkages (Figure 2). This compound is a non-reducing sugar that does not react with amino acids or proteins, making it unaffected by Maillard reactions. When incorporated into materials prior to freezing, trehalose has shown an ability to prevent ice crystallization damage to the molecule. This phenomenon has been exploited to increase the shelf life of materials ranging from foods to probiotic preparations. The role of trehalose is not limited to extending shelf life. It has been shown that mice supplemented with 2% trehalose had reduced symptoms of Huntington disease (Tanaka et al., 2004). Huntington’s disease is a rare, hereditary neurological illness characterized by sporadic and involuntary muscle movements. The disease affects approximately 1 in 10,000 people.

Carbohydrate-related diseases

The role of carbohydrates in health and disease is coming out of a blur and into focus, but we are only observing the infancy of this field of study. In 2002, a Harvard researcher proposed that an immune response to the complex carbohydrate glycosaminoglycan (GAG) is a potential cause of rheumatoid arthritis (Wang and Roehrl, 2002). Rheumatoid arthritis is a systemic autoimmune disease of connective tissue; and GAGs are a major component of that tissue. This study was unique in that it demonstrated a direct link between human disease, carbohydrate antigens and the immune system.

Congenital disorders of glycosylation (CDG), also known as ‘carbohydrate-deficient glycoprotein syndrome,’ are a group of inherited disorders where many glycoproteins are deficient in the carbohydrate fraction of the compound. Adults and children with CDG have varying degrees of disabilities such as speech and cognitive difficulties, poor balance and impaired motor skills. Several human diseases are a result of faulty carbohydrate metabolism, the most common of these being diabetes, a condition characterized by abnormally high levels of blood glucose from a failure in glucose transport from the blood into the cells. Another condition related to errors in carbohydrate metabolism is Tay-Sachs disease, an inherited disorder caused by a recessive
defect in the gene encoding for hexosaminidase A. This leads to an unusual accumulation of ganglioside GM₃ in the central nervous system. Characteristics of the disease include blindness, seizures, a degeneration of motor and mental function with early death in childhood. While most of the defined diseases of sugar metabolism are relatively rare, many of the so-called genetic diseases with unknown causes may be caused by errors in glycosylation because of the importance of glycosylation in cell-to-cell interaction and the role of glycomics in the immune system.

**Carbohydrates, cell-to-cell communications and defense against pathogens**

Carbohydrates are important surface entities of animal cells that function in a variety of ways to influence cell-to-cell communication, impact the immune system and allow bacterial attachment to the host. These complexed molecules project from the cell surface and form the antigenic determinants of certain cell types. One of the classical examples of this antigenicity is blood type in humans. The ABO blood group antigens are glycoproteins on red blood cells. Small differences in the terminal sugar residues distinguish the A and B blood-group antigens (Kuby, 1994; Figure 3) Mannose binding protein (MBP) is an integral part of the immune system. MBP in the serum can bind to terminal mannose groups on the surface of bacteria and interact with two serine proteases (MASP and MASP2), which ultimately lead to antibody independent activation of the classical pathway of the immune system (Roitt *et al.*, 1998).

Bacterial infection is due in many cases to the ability of the bacteria to recognize host cell surface sugars and use specific receptors that allow them to attach, colonize, and in the case of pathogens, cause disease in the animal. Mannose-specific adhesins (the binding entity on the surface of bacterial cells) are utilized by many gastrointestinal pathogens as a means of attachment to the gut epithelium. One way to prevent pathogens from causing disease is to prevent them from attaching to the epithelial cells in the gut. Early studies using mannose in the drinking water of broiler chicks demonstrated that this therapy could reduce colonization rate of *Salmonella typhimurium*. Purified mannose and a complex sugar called mannan oligosaccharide (MOS) have been successfully used to prevent bacterial attachment to the host animal by providing the bacteria a mannose-rich receptor that serves to occupy the binding sites on the bacteria and prevent colonization in the animal.

Several studies have been conducted examining the role of mannans and their derivatives on binding of pathogens to epithelial cells in the gastrointestinal tract. *E. coli* with mannose-specific lectins did not attach to mammalian cells when mannose was present (Salit and Gotschlich, 1977). Spring and coworkers (2000) used a chick model to demonstrate that MOS (Bio-Mos®) could significantly reduce the colonization of *Salmonella* and *E. coli*. Animal trials in other species show similar benefits in reducing colonization rates.

![Figure 3. Differences in the terminal sugar residues distinguish the A and B blood group antigens.](image-url)
pathogen concentrations. In dogs, as well as in poultry, reductions in fecal clostridial concentrations have also been noted with Bio-Mos® supplementation (Finucane et al., 1999; Strickling, 1999).

Fructo-oligosaccharides (FOS) have been investigated for nutritional manipulation of the gastrointestinal tract to inhibit pathogens. The principle behind the use of FOS involves the structure and bonding of the fructose molecules. Purified preparations of FOS have been shown to provide a nutrient source for beneficial bacteria such as bifidobacteria and certain lactobacilli. By supporting the growth of the beneficial bacteria it is thought that this will provide an in situ competitive exclusion (CE) effect, thus improving animal health. However, it seems important that the concentration of non-complexed fructose molecules be kept to a minimum in order for this oligosaccharide to be successful. Oyarzabal and coworkers (1995) found that Salmonella spp. could not use a purified FOS preparation for growth but were able to utilize a commercial preparation of FOS. The authors suggest the use of lactic acid bacteria in combination with FOS as a feasible approach to control Salmonella. Other studies have demonstrated a reduction in Salmonella concentrations in birds challenged with S. typhimurium with and without FOS and a CE culture. FOS alone had little effect on Salmonella exclusion when FOS was administered after infection, but FOS in combination with a defined CE product had an additive effect on Salmonella exclusion especially when used as a prophylactic prior to infection (Bailey et al., 1991). However consistent response of animals to FOS supplementation is a problem and may affect other as yet undefined interactions. Waldroup and coworkers (1993) found that supplemented broilers with 0.375% FOS had few consistent effects on production parameters or carcass Salmonella concentrations. These authors also caution of possible antagonism between FOS and BMD.

Human data for FOS are much more consistent. Hidaka et al. (1986) found that consumption of 8 g FOS/day increased numbers of bifidobacteria, improved blood lipid profiles and suppressed putrefactive substances in the intestine.

Glycomics also plays a vital role in viral diseases. The influenza virus infects by first attaching to a cell surface carbohydrate called sialic acid. This attachment ‘opens the door’ of the cell and allows the virus to replicate within. The commercial drugs Tamiflu and Relenza shorten the duration of the flu by binding to the active site of an enzyme produced by the virus that frees the virus from the sialic acid. By tying up this enzyme, the virus cannot easily spread and infect other cells (Schmidt, 2002). There are also data examining a novel anti-human immunodeficiency virus (HIV) protein. This protein, called actinohivin, binds to a glycoprotein on various HIV strains and simian immunodeficiency virus (SIV) inhibiting viral entry into cells by binding to this envelope glycoprotein. Further investigation showed that only yeast mannan can inhibit the binding of actinohivin to these viruses. These results demonstrate that the mannose saccharide chains of the virus glycoprotein are the molecular targets of the anti-HIV activity of actinohivin (Chiba et al., 2004). Sulfated galactomannans also demonstrate in vitro and in vivo activity against the flaviviruses, yellow fever virus and dengue virus (Ono et al., 2003). West Nile virus has also gained a strong foothold in the United States, affecting birds, horses and man. N-linked sugars with mannose residues on the cell membrane protein were found to be important in West Nile virus binding to the cell (Chu and Ng, 2003).

The future of the science of glycomics seems enormous at this time. While mannan oligosaccharide is currently being used to improve health and production of animals, there are enormous possibilities to use other sugars as possible agents against pathogen infection. Table 1 shows a partial summary of scientific studies examining bacterial adhesins.

### Table 1. Carbohydrate adhesins of various bacterial strains1.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Expressing mannose adhesins (% of adhesins examined)</th>
<th>Other carbohydrate adhesins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter coli</td>
<td>0</td>
<td>Glucose</td>
</tr>
<tr>
<td>Campylobacter jejuni²</td>
<td>0</td>
<td>Glucose</td>
</tr>
<tr>
<td>Clostridium spp.²</td>
<td>0</td>
<td>Galactose, glucose, lactose</td>
</tr>
<tr>
<td>Edwardsiella ictaluri</td>
<td>100</td>
<td>Not known</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>100</td>
<td>Not known</td>
</tr>
<tr>
<td>Escherichia coli²</td>
<td>53</td>
<td>Fucose, galactose, glucose</td>
</tr>
<tr>
<td>Fusobacterium spp.</td>
<td>0</td>
<td>Galactose, lactose, raffinose</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>0</td>
<td>Galactose, glucose</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>100</td>
<td>Glucose</td>
</tr>
<tr>
<td>Salmonella spp.²</td>
<td>64</td>
<td>Fucose, galactose</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>100</td>
<td>Not known</td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>75</td>
<td>Fucose</td>
</tr>
<tr>
<td>Streptococcus bovis</td>
<td>0</td>
<td>Glucose</td>
</tr>
<tr>
<td>Streptococcus suis</td>
<td>0</td>
<td>Galactose</td>
</tr>
</tbody>
</table>

1Summarized from Mirelman et al., 1980; 1986; Ofek et al., 2003

2In vivo data have shown reductions in these populations with Bio-Mos®.
Conclusions

To say that carbohydrates are involved in virtually every aspect of biology is not an understatement. Finding ways to exploit this knowledge is the current challenge. The vast array of possibilities that exist with polysaccharide structure and function make glycomics a science that may well pass on to future generations. We have only scratched the surface, but we have a better understanding of arthritis, how the immune system works in identifying invasion, how certain congenital disorders debilitate and we have used our limited knowledge to take advantage of the ‘sweet tooth’ of pathogens to control infection. It brings a whole new meaning to ‘a spoonful of sugar helps the medicine go down’.

References


Focus on Poultry
Selenium sources and selenoproteins in practical poultry production

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Introduction

Nutritional needs of food animals must be met by provision of nutrients from plants, soil, and even from prey animals. Many of the potential nutrients are minerals that exist in chemical complexes that are not readily available. This condition is advantageous for animals because many of the minerals can be toxic, but nutritionists have demonstrated that a large number of minerals, either as macronutrients or as micronutrients, are required for normal growth and development of animals including humans. The macronutrients consist of minerals such as calcium, phosphorus, sodium, and potassium because these are involved in structural integrity of the body and in homeostatic mechanisms. Micronutrients, often called trace nutrients, include minerals such as magnesium, manganese, zinc, iron, copper, and there are ultramicronutrient minerals such as molybdenum, selenium, iodine, cobalt, chromium and even vanadium. The trace minerals as either micro- or ultramicronutrients function as parts of proteins, hormones, enzymes, or as co-factors that activate specific enzymes.

Nature’s provision of nutrients to the inhabitants of the earth is a process that can be very variable. Although common foodstuffs such as corn, wheat, barley, soybeans, or oats are now grown around the world, they are not the same world wide. In the case of foodstuffs that provide selenium, some can be enriched as the result of high concentrations of selenium in the soil. In contrast, some foodstuffs can have very low levels of selenium because the soil in which they are grown had very low concentrations. This situation requires that supplemental selenium be provided via the manufactured feed to ensure optimal performance of food production animals.

Among the ultramicronutrients, selenium may hold the distinction of being the most difficult to understand. It can exist in four valence states: -2 (hydrogen selenide, sodium selenide, dimethyl selenide, trimethyl selenium, and selenoamino acids such as selenomethionine), 0 (elemental selenium), +4 (selenium dioxide, selenous acid, and sodium selenite), and +6 (selenic acid and sodium selenate), and depending upon its valence state and water solubility, the gastrointestinal absorption rate can be affected. Selenium can be toxic in valence states –2, +4 and +6, but in appropriate trace levels in feed or drinking water, selenium in valence states –2, +4 and +6 can also serve in several essential roles in maintenance of the body’s homeostatic condition.

Forms and availability of selenium used in animal production

Selenium supplementation of poultry rations is now a routine procedure. Since 1974, when the US Food and Drug Administration (FDA) approved selenium as a feed supplement, sodium selenite has become the traditional source for dietary supplemental selenium for poultry and livestock (Leeson and Summers, 1991). Other inorganic sources of selenium are sodium selenate and calcium selenate (Ecchevarria et al., 1988a,b). Poultry obtain all of their selenium through their feed. The commonly used feed ingredients, such as cereal products (Burk, 1976) and meals of fish, poultry and meat (Levander 1986; Cai et al., 1995), contain selenium almost exclusively as organic compounds such as the naturally occurring selenoamino acids (selenomethionine and selenocysteine). The selenoamino acids are incorporated into protein,
principally as selenomethionine and selenocysteine, and constitute 50 to 80% of the total selenium in plants and grains (Butler and Peterson, 1967).

Estimates of bioavailability of selenium for poultry vary considerably depending upon which criteria are used for the evaluation. Scott and Thompson (1971) determined that there was a linear relationship in tissue deposition of dietary inorganic selenium up to 0.3 mg/kg. Cantor et al. (1975a,b) have reported diverse and variable results when exudative diathesis or pancreatic fibrosis were used to evaluate selenium bioavailability. In both studies, plant-based selenium sources were superior to inorganic sources, but there were low protective values associated with plant-based organic selenium in terms of protection against development of exudative diathesis (Cantor et al., 1975a). In contrast, Osman and Latshaw (1976) reported that selenomethionine was at least equivalent to sodium selenite in the protection of the chick against exudative diathesis. Cantor et al. (1975b) reported high availability of selenomethionine in protection of the chick against pancreatic fibrosis. Echevarria et al. (1988a,b) indicated that sodium selenite, sodium selenate, and calcium selenate are absorbed equally well by chicks based upon tissue distributions of selenium after short term feeding trials with high levels of the inorganic selenium sources. Echevarria et al. (1988a,b) demonstrated that selenium concentrations ranged from highest to lowest in kidney, liver, muscle, and plasma, respectively. Increasing levels of dietary inorganic selenium were associated with greater tissue concentrations. The increasing tissue concentrations of selenium were time dependent with liver showing weekly increases in selenium concentrations whereas selenium reached a plateau within seven days of feeding in kidney, muscle, and plasma. In a study conducted with rats, Vinson and Bose (1987) found that organic selenium from yeast was more available to blood and liver proteins than from either sodium selenite or an amino acid-selenium chelate (Table 1).

Table 1. Bioavailability of selenium forms in the inorganic sodium selenite, amino acid chelate, and organic selenomethionine in yeast (Vinson and Bose, 1987).

<table>
<thead>
<tr>
<th>Form of selenium</th>
<th>Tissue</th>
<th>Bioavailability, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium selenite</td>
<td>Blood</td>
<td>100</td>
</tr>
<tr>
<td>Chelated amino acid</td>
<td>Blood</td>
<td>60</td>
</tr>
<tr>
<td>Yeast</td>
<td>Blood</td>
<td>138</td>
</tr>
<tr>
<td>Sodium selenite</td>
<td>Liver</td>
<td>100</td>
</tr>
<tr>
<td>Chelated amino acid</td>
<td>Liver</td>
<td>88</td>
</tr>
<tr>
<td>Yeast</td>
<td>Liver</td>
<td>147</td>
</tr>
</tbody>
</table>

The availability of selenium from different sources such as selenomethionine or inorganic selenium from selenite or selenate may be affected by numerous factors. Cantor and Johnson (1985) concluded that selenium was made more available in diets that were low in protein, possibly as a result of decreased total methionine in the diet. The kind and quality of fish meal that has been used in poultry diets also influences the availability of selenium for poultry (Miller et al., 1972; Whitacre and Latshaw, 1982). Furthermore, intestinal absorption of selenium in foodstuffs varies by species, and bioavailability varies according to the form that is eaten (Gabrielsen and Opstevedt, 1980; Douglas et al., 1981; Ringdal et al., 1985; Wen et al., 1997; Schen et al., 1997). Schen et al. (1997) reported that selenomethionine and sodium selenate are more diffusible than selenocysteine and sodium selenite, explaining the higher absorption rate for selenomethionine and sodium selenate. However, utilization of selenium from sodium selenate can be quite variable because a significant fraction may be lost in urine before it can be incorporated into body tissues (Xia et al., 1992).

Bioavailability of selenium has been estimated by a tissue residue approach by numerous investigators. Miller et al. (1972) found selenomethionine to be more available to chicks than selenium from sodium selenite or selenium from fish products. Cantor et al. (1982) observed significantly more selenium from selenomethionine in muscles of turkey poults than selenium from inorganic selenium sources. However, Cantor and Tarino (1982) showed that availability of selenium from selenite was greater than from selenomethionine when plasma glutathione peroxidase (GSH-Px) activity was used an indicator. Moksnes and Norheim (1986) also demonstrated greater tissue concentrations of selenium and GSH-Px activity in selenomethionine-fed chickens compared with sodium selenite-fed chickens. There is no question that selenomethionine will increase tissue concentrations of selenium as compared with selenium deposition from inorganic selenium.

When the FDA approved selenium supplements for poultry and swine in 1974, the animal industries settled on the use of inorganic selenium forms rather than the organic selenoamino acids. The primary reason for that decision was based on cost of the selenium supplements and lack of information on selenomethionine. The inorganic forms were cheap and the organic forms were expensive. Without a doubt, the use of inorganic selenium supplements in feeds has improved the performance of all classes of
commercial poultry. In modern high-yielding poultry, which have higher metabolic rates and different nutritional needs compared with poultry from 30 years ago, there is a need to reassess nutrient requirements. Inorganic selenium has some problems associated with its use. Among those problems are the minimal levels of selenium in meat proteins and the potential for toxicity if too high a dietary level of inorganic selenium is provided to chickens. Thus, a need to revisit organic selenium as a feed supplement for poultry is apparent.

After many years of laboratory and field research, a source for natural organic selenium (Sel-Plex®, Alltech Inc., Nicholasville, KY), was approved for use in the poultry industry in the US by the FDA (Federal Register, 2000; 2002). Sel-Plex® provides a broad spectrum of selenium compounds (Kelly and Power, 1995), but selenomethionine in the selenium-enriched yeast cellular protein component is the primary form of selenium in Sel-Plex®. The organic selenium profile in Sel-Plex® is similar to the organic selenium profile in plants and grains (Kelly and Power, 1995). The organic selenium in Sel-Plex® is readily available and will be absorbed actively (Mahan, 1994; 1995) from the intestine via the Na+-dependent methionine transport system (Spencer and Blau, 1962) while sodium selenite is absorbed passively by diffusion from the intestinal tract (McConnell and Cho, 1965; Schrauzer, 2001).

Even though organic selenium in Sel-Plex® is a superior source of selenium for poultry production (Edens, 1996; 2002; Edens and Sefton, 2002; Edens et al., 2002), sodium selenite is still used as the principal source of selenium in animal feeds. This seems paradoxical because sodium selenite has a documented pro-oxidant influence in all animals tested including humans (Hafeman et al., 1974; Csallany and Menken, 1986; Spallholz, 1997; Terada et al., 1999).

Selection of the best form of organic selenium as a feed supplement

Based on natural availability of organic selenium in plant–based foodstuffs and its superior bioavailability, it should be easy to decide to provide organic feed supplements, rather than the pro-oxidative sodium selenite, to food producing animals. However, the choices among selenium supplements available to feed compounders and commercial nutritionists are rather large and diverse in their composition. Schrauzer (2001) has reported that there are quality concerns associated with many selenium supplements. Some yeast products do not contain organic selenium. Instead they are made with inorganic sodium selenite or sodium selenate. Some feed supplements do not tell which form of selenium is provided. Some products are made with selenium proteinates or selenoamino acid chelates. Schrauzer also pointed out that vitamin supplements for humans are often formulated with vitamin C and that over time, the selenium is reduced to elemental selenium and is not available.

Thus, it is clear that the compounders and commercial nutritionists have had a difficult task in making an informed decision concerning the appropriate product to use for supplementation of organic selenium. There are many companies around the world that produce organic selenium yeast products. Each manufacturer has claimed superior performance of food animals fed their product. However, little is known about speciation of selenium in selenized yeast products. In a recently published article, investigators, using state-of-the-art methodologies, studied the fractionation of selenium in yeast into different classes of chemical species and determined the true speciation of protein-incorporated selenium in individual yeast proteins characterized by unique amino acid sequences (Encinar et al., 2003). In this study, they used three commercial yeast products. They quantified water soluble selenium, water insoluble polysaccharide-bound selenium, water-insoluble protein-bound selenium, residual protein-bound selenium, and residual hydrolysable selenium (Table 2). Based on these percentage values, it is apparent that the profile of selenocompounds differs markedly, which indicates that all sources of ‘selenium yeast’ are not alike. Thus, as one begins to think about and then select a yeast product for dietary organic selenium supplementation for food producing animals, it is important to know about the availability of the selenium in the product.

<table>
<thead>
<tr>
<th>Selenium species in three selenized yeast products1.</th>
<th>A (%)</th>
<th>B (%)</th>
<th>C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water soluble Se</td>
<td>11.5</td>
<td>28.0</td>
<td>21.0</td>
</tr>
<tr>
<td>Water insoluble polysaccharide-bound Se</td>
<td>15.5</td>
<td>27.0</td>
<td>72.0</td>
</tr>
<tr>
<td>Water insoluble protein-bound Se</td>
<td>19.0</td>
<td>38.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Residual protein-bound Se</td>
<td>38.0</td>
<td>6.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Residual hydrolysable Se</td>
<td>16.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

1Percentage values based on Encinar et al., 2003
Why is selenium an essential nutrient?

The essentiality of selenium as an ultramicronutrient in the daily nutrition of mammals was demonstrated by Schwarz and Foltz (1957). Their discovery that selenium deficient rats would suffer hepatic necrosis was rapidly followed by the discovery that selenium was essential in poultry (Schwarz et al., 1957). At Lederle Laboratories, Pearl River, New York, Patterson et al. (1957) made an independent observation that exudative diathesis in chickens was prevented when selenium was added to the feed. It was not until 1973 that the beneficial effects of selenium could be ascribed a biochemical function when Rotruck et al. (1973) and Flohé et al. (1973) discovered that it was an essential component of an important antioxidant enzyme, glutathione peroxidase (GSH-Px). Continued study of GSH-Px has shown that there are at least six isoenzyme forms in various organs and tissues in mammalian species (Table 3). Corresponding evidence is not available in avian species, but empirical data from studies with selenium in poultry suggest that several functional GSH-Px isoenzymes and other selenoproteins are also present in avian species. This would suggest that at least for GSH-Px and some of the other selenoproteins, the active selenocysteine site on the enzyme/protein has been conserved. Conservation of active sites may not be limited to vertebrate animals. Indeed, in a parasite, the blood fluke Schistosoma mansoni, phospholipid-hydroperoxide GSH-Px has been discovered (Maiorino et al., 1996). Thus, selenium has a special function in antioxidant control mechanisms in animal species.

Mertz (1987) suggested that selenium is unique among the essential trace elements especially in the manner in which its deficiency is expressed. Mertz (1987) stated that a selenium deficiency in mammals was likely to be manifest in second generation progeny. Selenium deficient rats with clinical signs die within a few weeks if they become vitamin E deficient (Schwarz, 1951), but if rats in the first generation of selenium deficiency are force-fed high levels of fat, they die within a few hours (Schwarz, 1954). These observations by Schwarz and by Mertz pointed to the complex interaction between selenium and vitamin E and established that there was a need for adequate levels of both in diets of humans and food animals.

A survey of the literature readily reveals that selenium, through the selenoproteins, influences numerous physiological and biochemical functions that are necessary for maintenance of homeostasis in all animals. Edens (1996) listed a number of problems associated with selenium deficiency. Selenium deficiency can manifest itself in many diseases and dysfunctions such as liver necrosis, muscular dystrophy, microangiopathy, exudative diathesis, pancreatic fibrosis, poor feathering, retained placenta, mastitis, cystic ovaries, general unthriftiness, Keshan disease, Kashin-Beck disease, cancer, numerous heart diseases, immune deficiencies, reduced fecundity, and many others and can affect humans and food production animals alike. These listed problems show us that no animal is exempt from selenium deficiency syndromes. Therefore, one can confidently state that selenium is essential and must be provided if natural intake is below optimal requirements.

Selenium in body proteins

Schrauzer (2003) reported that selenomethionine is incorporated into body proteins in place of methionine, providing a means for reversible storage of selenium in organs and tissues. This property of selenomethionine is not shared with any other selenoamino acid, which points to a specific physiological function of selenomethionine (Schrauzer, 2003). In fact, Schrauzer (2003) has asserted that all needed metabolic forms of selenium can be produced from selenomethionine, and it, therefore, meets all the criteria for an essential amino acid. The situation associated with the use of inorganic selenium is different because inorganic selenium from selenite or selenate results in very limited nonspecific insertion of selenocysteine into protein but serves as a substrate for synthesis of selenocysteine for specific insertion into selenoproteins (Hawkes et al., 1985a,b; 2003). Selenium from sodium selenite or selenate might also form a selenotrisulfide (-S-Se-S-) bond (Ilian and Whanger, 1989) that is subject to rapid oxidation and release from protein. The selenotrisulfide bond is not formed with selenomethionine.

Cummins and Martin (1967) and Latshaw and Osman (1976) have demonstrated that selenium derived from selenite was easily released from animal protein subjected to alkaline dialysis whereas selenium from selenomethionine was retained as a part of the protein. Ort and Latshaw (1978) have shown that even when toxic levels of selenomethionine or sodium selenite are fed to laying hens, egg levels and body tissue levels return to normal within two to four weeks after cessation of
feeding toxic levels of selenite selenium, but a longer time is required for birds fed selenomethionine. Selenium from selenite, therefore, appears to interact loosely with the cysteine thiol group and selenium in selenomethionine is molecularly integrated into protein. The stored form of organic selenium as selenomethionine is in a non-functional state, i.e. not used immediately for formation of biologically functional selenoproteins (Mahan, 1994; 1995). Since selenium in selenomethionine is better retained than inorganic selenium, the ultimate metabolism of organic selenium with liberation of selenide that enters the pathway for selenocysteine synthesis for incorporation into functional selenoproteins appears to be more efficient. As a consequence of the large pool of stored selenium in protein, in times of oxidative stress, body protein can be degraded rapidly providing more than adequate concentrations of organic selenium that can be used for synthesis of specific selenoproteins.

Constant provision of a readily available source of organic selenium from body proteins for synthesis of selenoproteins is a unique and continuous process. Mitch and Goldberg (1996) have shown that most proteins are degraded within a few hours after their synthesis. Functional proteins such as enzymes have a more rapid degradation rate than do structural proteins such as muscle protein. Consumption of diets containing organic selenium results in a high rate of selenomethionine incorporation in muscle protein. As the muscle protein is replaced, the stored selenomethionine is released into the free amino acid pool from which it is made available for selenoprotein synthesis. In times of oxidative stress, the proteasome, a cytosolic organelle, increases the rate of protein degradation and increases amino acid availability for synthesis of other proteins such as the selenoproteins. In fast growing animals such as the high-yielding commercial broiler lines, it is important to have a readily available source of selenium to be used in selenoprotein synthesis.

**Selenium incorporation into selenoproteins**

The interest in the antioxidant properties of selenium has been facilitated by the fact that the active site(s) on the various selenoproteins, which often act in antioxidant systems, contain selenocysteine- the proclaimed 21st amino acid. The locus of selenocysteine in selenoproteins involves use of a stem-loop structure in the 3′ non-translated region of the mRNA that designates selenocysteine insertion at the UGA codon instead of chain termination (Berry *et al.*, 1991a,b; 1993).

In order for selenocysteine to be incorporated into specific selenoproteins, there is a requirement for selenocysteine-β-lyase reaction with free selenocysteine that causes the release of selenide in the presence of reducing agents (Sunde, 1990; Burk, 1991). Another selenoenzyme, selenophosphate synthetase, using selenide and serine as substrates, phosphorylates selenide to form selenophosphate. The selenophosphate is made available to a unique seryl-tRNA$_{Sec}$ that is recognized by selenocysteine synthetase. Under the influence of selenocysteine synthetase, that converts seryl-tRNA$_{Sec}$ to selenocysteyl-tRNA$_{Sec}$, the selenide in selenophosphate is co-translationally incorporated into selenocysteine. Then, selenocysteyl-tRNA$_{[Ser]^{Sec}}$, which recognizes the specific UGA codon in the selenoprotein-mRNA, inserts the new, co-translationally synthesized selenocysteine into the specific selenoprotein (Burk, 1991). The base triplet UGA that normally functions as a stop codon (Amberg *et al.*, 1996) encodes this process of selenocysteine insertion at its appropriate site in the peptide. The selenocysteine insertion also requires a specific mRNA, an elongation factor, GTP, and the selenocysteine insertion sequence (SECIS) that all interact at the ribosome to read the UGA selenocysteine codon (Low and Berry, 1996).

Thus, organic selenium must be converted from its original organic form (-2 valence) to the inorganic selenide (also -2 valence) form then back to an organic form (-2 valence) to fulfill its biological function (Arthur, 1997). This conversion is crucial with regard to synthesis of selenoproteins because it has been reported that 30 to 80% of the selenium in the body may be selenocysteine (Hawks *et al.*, 1985a). Nevertheless, selenomethionine is a highly available substrate for many proteins and can substitute non-specifically for methionine in their structure (Daniels, 1996). In cases of selenomethionine supplementation to feeds, it can be demonstrated that 40 to 50% of total body selenium, as selenomethionine, can be found in muscle protein (Daniels, 1996). Natural selenocysteine also can be substituted non-specifically for cysteine in many proteins, but it is not incorporated directly into specific selenoproteins (Sunde, 1990; Daniels, 1996).

Animals cannot synthesize selenomethionine, the primary selenoamino acid, directly from selenite or selenate forms of inorganic selenium (Cummins and
Selenium functions through selenoproteins

In mammals there are at least 18 known (Hatfield and Gladyshev, 2003) and possibly more genes that encode selenoproteins (Behne et al., 1997). Recent evidence strongly suggests that there are at least 25 selenoproteins in the mammalian selenoproteome (Kryukov et al., 2003), and this would suggest more genes that encode selenoproteins. The known selenoproteins have numerous functions, but many of the selenoproteins still have unknown functions (Table 3). Furthermore, it is not known if all animals possess equivalent proteins. An excellent review of the functions of selenoproteins in farm animals was presented by Jacques (2001) who pointed out that selenium must exert its greatest influences through selenoproteins.

In poultry there has been limited research to ascertain the presence and function(s) of the various selenoproteins. It appears from the few publications on avian selenoproteins that their functions are generally similar to the corresponding protein(s) in mammals (Petovich and Podorozhnaia, 1981; Ilian and Whanger, 1989; Snityns’kyi and Antoniak, 1994). Of the selenoproteins studied in chickens, it has been reported that there are two deiodinases: Type I that functions in the liver to convert thyroxin (T4) to triiodothyronine (T3), and Type III that functions to convert T3 to rT3 and T3 to T2 (May, 1989; Van der Geyten et al., 1997). There are numerous reports on GSH-Px activity(ies) in chickens, and those reports suggest that the GSH-Px enzymes function similarly to those found in mammals because of their specificity for reduction of hydrogen peroxide and lipid peroxides (Stadtman, 1980; Pablos et al., 1995a,b; 1998). Recent evidence has shown that chickens express phospholipid-hydroperoxide GSH-Px (Kong et al., 2003), supporting observations of its presence in several chicken and turkey tissues (Surai et al., 1998a, b). The chicken is different from most mammals because it also expresses a monomeric GSH-Px in its tissues (Miyazaki and Motoi, 1992; 1996). Finally, thioredoxin reductase (TrxR) activity has been reported for the chicken (Smith and Levander, 2001; 2002), and it was determined that the activity of chicken TrxR is very much less than mammalian TrxR. Tissue distribution of chicken TrxR has not been determined yet, but preliminary data will be presented herein. The presence of other selenoproteins can be inferred from current and previous studies; but based on the literature, it is possible that the chicken will express selenoproteins similar to mammals.

Edens (2001) reported that broiler chickens given a Sel-Plex® supplement in the feed were more resistant to enteropathogenic E. coli enteric infection than chickens that had no supplemental selenium. The broilers given Sel-Plex® had body weights and feed conversions that were similar to the birds with no supplemental selenium and not challenged with the bacteria (Table 5). Mortality of E. coli-challenged Sel-Plex®-fed broilers was 59% less than mortality in E. coli-challenged broilers given no supplemental selenium (Table 5). Additionally, the GSH/GSSG ratios in broilers given Sel-Plex® were less than those that were not given supplemental selenium (Table 6). This suggested that the GSH/GSH-Px system was working more efficiently in broilers fed Sel-Plex®. This conclusion was supported by the observation that there was less heat shock protein (hsp) 70 in the intestines of Sel-Plex®-fed birds compared with those given no supplemental selenium (Figure 1).

Sulfur-containing amino acids (cysteine and methionine) are susceptible to oxidation. The sulfhydryl groups of cysteine residues of proteins are normally maintained in a reduced state or they can be oxidized by thioredoxins in the cytoplasm (Okamoto et al., 1999). During oxidative stress, thiol-disulfide exchange occurs between cysteine residues in protein and oxidized glutathione (GSSG). This process serves as a redox-dependent regulator of various protein functions (Fratelli et al., 2002). The oxidized sulphydryl in methionine can be reduced by the methionine sulfoxide reductases (Grimaud et al., 2001) and might serve in an antioxidative capacity (Levine et al., 1996; Stadtman et al., 2002). These mechanisms are interrelated because the heat shock proteins elevate reduced glutathione levels by promoting an increase in glucose-6-phosphodehydrogenase activity (Previle et al., 1999). The heat shock proteins are normally induced during
Table 3. Selenoproteins and their role in the maintenance of homeostasis in animals.

<table>
<thead>
<tr>
<th>Selenoproteins</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glutathione peroxidases (GSH-Px)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH-Px-1 (cytosolic)</td>
<td>Reduces reactive molecules and free radicals; complements action of vitamin E</td>
<td>Rotruck et al., 1973; Flohé et al., 1973</td>
</tr>
<tr>
<td>GSH-Px-2 (gastrointestinal)</td>
<td>Antioxidant; reduces ingested lipid hydroperoxides</td>
<td>Chu et al., 1993</td>
</tr>
<tr>
<td>GSH-Px-3 (plasma)</td>
<td>Unknown; selenium carrier</td>
<td>Takahashi et al., 1987</td>
</tr>
<tr>
<td>Phospholipid hydroperoxide</td>
<td>Directly reduces phospholipids and cholesterol</td>
<td>Ursini et al., 1985</td>
</tr>
<tr>
<td>GSH-Px- sperm nucleus</td>
<td>Protamine thiol peroxidase; responsible for disulfide cross-linking; necessary for sperm maturation and male fertility</td>
<td>Behne et al., 1988, 1997</td>
</tr>
<tr>
<td>GSH-Px-6</td>
<td>Unknown; homologue of GPx-1</td>
<td>Kryukov et al., 2003</td>
</tr>
<tr>
<td><strong>Thioredoxin reductases (TrxR)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytosolic TrxR (TrxR1)</td>
<td>Reduces protein thiols and thioredoxin; provides reducing equivalents to several redox-dependent systems</td>
<td>Tamura and Stadtman, 1996</td>
</tr>
<tr>
<td>Testicular TrxR (TrxR2)</td>
<td>Reduces protein thiols and thioredoxin; provides reducing equivalents to several redox-dependent systems</td>
<td>Gasdaska et al., 1999; Lee et al., 1999; Miranda-Vizuete et al., 1999; Watabe et al., 1999</td>
</tr>
<tr>
<td>Mitochondrial TrxR (TrxR3)</td>
<td>Reduces protein thiols and thioredoxin; provides reducing equivalents to several redox-dependent systems</td>
<td>Sun et al., 1999</td>
</tr>
<tr>
<td><strong>Iodothyronine deiodinases (ID)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ID Type-I</td>
<td>Converts thyroxin (T4) to tri-iodothyronine (T3)</td>
<td>Behne et al., 1988, 1990; Arthur et al., 1990; Berry et al., 1991a</td>
</tr>
<tr>
<td>ID Type-II</td>
<td>Converts T4 to T3 and rT3 to T2</td>
<td>Croteau et al., 1996; Salvatore et al., 1996</td>
</tr>
<tr>
<td>ID Type-III</td>
<td>Antioxidant in brain; converts T3 to rT3 and rT3 to T2; protects developing brain from excess T3</td>
<td>Kaplan, 1986; Croteau et al., 1995; Mortimer et al., 1996</td>
</tr>
<tr>
<td><strong>Other selenoproteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sel H</td>
<td>Unknown</td>
<td>Kryukov et al., 2003</td>
</tr>
<tr>
<td>Sel I</td>
<td>Unknown</td>
<td>Kryukov et al., 2003</td>
</tr>
<tr>
<td>Sel K</td>
<td>Unknown</td>
<td>Kryukov et al., 2003</td>
</tr>
<tr>
<td>Sel M</td>
<td>Unknown</td>
<td>Kryukov et al., 2003</td>
</tr>
<tr>
<td>Sel N</td>
<td>Unknown</td>
<td>Kryukov et al., 2003; Lescure et al., 1999</td>
</tr>
<tr>
<td>Sel O</td>
<td>Unknown</td>
<td>Kryukov et al., 2003</td>
</tr>
<tr>
<td>Sel P</td>
<td>Selenium carrier; antioxidant</td>
<td>Herman, 1977; Motsenbocker and Tappel, 1982</td>
</tr>
<tr>
<td>Sel R</td>
<td>Methionine sulfoxide reductase; antioxidant activities and aging</td>
<td>Kryukov et al., 2003; Lescure et al., 1999; Moskovitz et al., 2001</td>
</tr>
<tr>
<td>Sel T</td>
<td>Unknown</td>
<td>Kryukov et al., 2003; Lescure et al., 1999</td>
</tr>
<tr>
<td>Sel U (SEC in chickens and fish; Cys-homologue in mammals)</td>
<td>Unknown</td>
<td>Catellano et al., 2004</td>
</tr>
<tr>
<td>Sel V</td>
<td>Unknown; homologue of Sel W</td>
<td>Kryukov et al., 2003</td>
</tr>
<tr>
<td>Sel W</td>
<td>Unknown; redox activity in muscles and other tissues</td>
<td>Vendeland et al., 1993; Allan et al., 1999</td>
</tr>
<tr>
<td>Sel X</td>
<td>Unknown</td>
<td>Kryukov et al., 2003; Lescure et al., 1999</td>
</tr>
<tr>
<td>15 kDa Selenoprotein</td>
<td>Unknown; found in T cells and prostate</td>
<td>Gladyshev et al., 1998</td>
</tr>
<tr>
<td>18 kDa Selenoprotein (mitochondrial)</td>
<td></td>
<td>Behne et al., 1988; Kyriakopoulos et al., 1996</td>
</tr>
<tr>
<td>Selenophosphate synthetase-2</td>
<td>Biosynthesis of selenocysteine used in selenoprotein synthesis</td>
<td>Low et al., 1995</td>
</tr>
</tbody>
</table>
Table 5. Performance of 42 days old male broiler chickens given a dietary supplement of Sel-Plex® and challenged with an enteropathogenic E. coli.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Blood (before heat)</th>
<th>Blood (after heat)</th>
<th>Liver (before heat)</th>
<th>Liver (after heat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGSH, µM/g Hb or µM/g tissue</td>
<td>Control</td>
<td>15.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2786&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1997&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Selenite</td>
<td>14.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2320&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2120&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Sel-Plex&lt;sup&gt;®&lt;/sup&gt;</td>
<td>13.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2430&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2297&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.65</td>
<td>0.65</td>
<td>254</td>
<td>254</td>
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<tr>
<td>GSH, µM/g Hb or µM/g tissue</td>
<td>Control</td>
<td>12.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2695&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1951&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Selenite</td>
<td>11.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2269&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2082&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>10.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2346&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2185&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
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<td>0.46</td>
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<td>249</td>
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<td>GSSG, µM/g Hb or µM/g tissue</td>
<td>Control</td>
<td>1.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Selenite</td>
<td>1.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Sel-Plex&lt;sup&gt;®&lt;/sup&gt;</td>
<td>1.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.15</td>
<td>0.15</td>
<td>14.7</td>
<td>10.7</td>
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<tr>
<td>GSH/GSSG</td>
<td>Control</td>
<td>7.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.84a</td>
<td>85.57&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>6.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>108.44&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td>Sel-Plex&lt;sup&gt;®&lt;/sup&gt;</td>
<td>7.52a</td>
<td>6.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.12a</td>
<td>38.74&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>SEM</td>
<td>0.28</td>
<td>0.37</td>
<td>16.27</td>
<td>21.31</td>
</tr>
<tr>
<td>GSHpx, mU/mg Hb or mU/mg protein</td>
<td>Control</td>
<td>9.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2198&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1632&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Selenite</td>
<td>58.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3602&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2936&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Sel-Plex&lt;sup&gt;®&lt;/sup&gt;</td>
<td>139.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>190.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3836&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3432&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>7.86</td>
<td>7.86</td>
<td>390</td>
<td>190</td>
</tr>
<tr>
<td>GR, mU/mg Hb or mU/mg protein</td>
<td>Control</td>
<td>2.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>187.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>163.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Selenite</td>
<td>2.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>169.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>159.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Sel-Plex&lt;sup&gt;®&lt;/sup&gt;</td>
<td>2.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>173.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>176.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>SEM</td>
<td>0.28</td>
<td>0.28</td>
<td>9.1</td>
<td>9.1</td>
</tr>
<tr>
<td>hsp70 (ng/mg total protein)</td>
<td>Control</td>
<td></td>
<td>4.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Selenite</td>
<td></td>
<td>4.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<td></td>
<td>Sel-Plex&lt;sup&gt;®&lt;/sup&gt;</td>
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<td>3.95&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>SEM</td>
<td>0.43</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>,<sup>b</sup>,<sup>c</sup>In a column, means with unlike superscripts differ significantly (P ≤ 0.05).

The data from these two different experiments suggest that in three tissues there might be three or four different GSH-Px, specifically the classical GSH-Px-1, GSH-Px-2, GSH-Px-3 and monomeric GSH-Px. Although, the data cannot predict which isoform was active/not active, it was apparent that the GSH/GSH-Px system in the chicken is highly responsive to selenium both as sodium selenite and as organic selenomethionine in yeast protein.

Edens, 2001). If the hypothesis presented here is correct, the selenium in selenomethionine and selenocysteine in proteins might be less reactive with oxidized GSSG, and the GSSG would be reduced more readily by glutathione reductase as postulated by Mahmoud and Edens (2003). As a result, when organic selenium is fed to broilers, less heat shock protein would be induced because the cell cytoplasm would be maintained in a more reduced status even in the face of stressors that promote oxidative distress (Table 4). Oxidative stress and protect sensitive sites on proteins. In the results of two studies presented here, we concluded that broilers given Sel-Plex® and exposed to either heat stress (Table 4) or enteric an E. coli challenge (Figure 1) performed better because they had lower concentrations of liver hsp70 than broilers given sodium selenite or no supplemental selenium.
Without a highly functional selenium-dependent antioxidant system, the modern broiler chicken would not be as productive. As an illustration, Edens (2001) presented performance data of broilers grown to 42 days of age. In that experiment Sel-Plex®-fed broilers had body weight improvement of 20 g over selenite supplemented broilers and a 50 g improvement over broilers with no selenium supplementation. Feed conversion in Sel-Plex®-fed broilers was improved 3 points over selenite-fed birds and 9 points over birds with no supplemental selenium.

### Monodeiodinase and triiodothyronine: influences on diverse functions and poultry performance

The thyroid hormone, triiodothyronine (T₃) has many important functions in all animals. In poultry, T₃ has the potential to affect the efficiency of conversion of food into energy and metabolic heat production. The mitochondria, the cell’s power generators, are affected directly by T₃. Rapid protein synthesis, mitochondrial gene transcription, and synthesis of proteins from genetic information are affected by T₃. These processes cause turnover of body proteins, increase free fatty acids for energy metabolism, and increase oxygen usage by cells in the body. Additionally, the cardiovascular system is stimulated to a higher level of activity to meet the tissue demands for increased oxygen caused by higher levels of T₃. Body temperature regulation is also partly under the control of circulating T₃ that influences mitochondrial activity in muscles. The circulating T₃ level is highly responsive to caloric intake and external temperature and is necessary for cold adaptation. If caloric intake increases or external temperature decreases, there is an increase in circulating T₃. Conversely, with

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**Table 6. Influence of Sel-Plex® on intestinal total glutathione (µM/g), oxidized glutathione (µM/g), reduced glutathione (µM/g), and the reduced:oxidized glutathione ratios in broiler chickens given a dietary supplement of organic selenium and challenged with an enteropathogenic E. coli.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total glutathione</th>
<th>Oxidized (GSSG)</th>
<th>Reduced (GSH)</th>
<th>Ratio R:O</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Selenium, no E. coli</td>
<td>18954 ± 1904ab</td>
<td>1601 ± 196c</td>
<td>13349 ± 1835b</td>
<td>8.32 ± 1.05a</td>
</tr>
<tr>
<td>No Selenium + E. coli</td>
<td>21896 ± 1971a</td>
<td>3047 ± 180d</td>
<td>19099 ± 1688a</td>
<td>7.30 ± 1.44ab</td>
</tr>
<tr>
<td>Sel-Plex®, no E. coli</td>
<td>17045 ± 1843b</td>
<td>2238 ± 174b</td>
<td>13322 ± 1627b</td>
<td>5.95 ± 1.01b</td>
</tr>
<tr>
<td>Sel-Plex® + E. coli</td>
<td>22500 ± 1971a</td>
<td>3313 ± 188c</td>
<td>18911 ± 1757a</td>
<td>5.71 ± 1.09b</td>
</tr>
</tbody>
</table>

abc In a column, means with unlike superscripts differ significantly (P ≤ 0.05).
starvation T3 decreases along with increased thyroxin (T4). The development and growth of animals is directly affected by T4 binding to thyroid receptors (TR) that bind to responsive elements on the nuclear DNA triggering gene transcription. This T4 function in all animals is extremely important in the developing embryo for growth and hatching efficiency and in the post-hatch chicken for growth. Recently, a report was given that sodium selenite actually inhibited the development of the T4-TR bond, but organic selenium did not inhibit that relationship (Brtko et al., 1997).

Involvement of thyroid hormones in feathering has long been recognized (Radi and Warren, 1938; Boone et al., 1950). Feeding thyroprotein increases feathering rate in slow feathering birds. In thyroprotein there is a predominance of thyroxin (T4), but in poultry, triiodothyronine (T3) is the most active thyroid hormone. It is known that T3 is intimately involved in feather development. A small quantity of T3 is produced in the thyroid, but the greatest quantity is converted from T4 to T3 in the liver by the selenium-dependent type I 5′-deiodinase enzyme (Berry et al., 1991a) that predominates in the chicken (May, 1989).

Experiments have been conducted to compare selenium from sodium selenite and from Sel-Plex® on feathering in broiler chickens (Edens, 1996; 2001). It was determined that organic selenium induced more rapid feathering in auto-sexing, slow feathering male chickens and in their normal feathering sisters (Edens, 2001). An evaluation of thyroid hormone levels in broiler chickens was made in an attempt to relate thyroid hormones to feathering (Edens, 2001). When organic selenium in Sel-Plex® was fed to broilers, the circulating percentage concentrations of T4 were lower and T3 concentrations were higher than in broilers given sodium selenite (Table 7). These observations suggest that organic selenium is more important than inorganic selenium in the early feathering of chickens and possibly other birds.

Edens (2001) reported that body weight of broilers fed organic selenium in Sel-Plex® was improved (70 g/bird and 20 g/bird, respectively) over that of broilers fed no supplemental selenium and those fed sodium selenite. Feed conversions of broilers fed organic selenium in Sel-Plex® was also improved (9 points and 3 points, respectively) over that of broilers fed no supplemental selenium and those fed sodium selenite. Part of the improvement in performance was due to the status of the metabolically active thyroid hormone in the broilers fed Sel-Plex® (Table 7). These observations were supported by the report of Jianhua et al. (2000), who examined hepatic 5′-deiodinase activity and thyroid hormone dynamics in broilers fed selenium deficient or selenium (selenite) adequate diets. They concluded that selenium deficiency depressed growth of broilers by inhibiting 5′-deiodinase activity, which in turn decreased the plasma concentration of T3.

The role of selenium in the immune system of chickens has received little interest to date. We have noted that fast growing, high yielding broilers fed selenite at 1.2 ppm Se showed early signs of selenium toxicity by exhibiting decreased body weight, increased liver weight, and a compromised immune system as indicated by decreased thymus and bursa of Fabricius weights and abnormally high delayed type hypersensitivity reactions to phytohemagglutinin antigen (PHA-P), but broilers fed Sel-Plex® at 1.2 ppm were not affected (Gowdy and Edens, 2003). Marsh et al. (1986) fed low levels of sodium selenite to chickens and observed increased growth of lymphoid organs including an increased thymus weight. Gowdy and Edens (2003) found that feeding sodium selenite (0.3 or 0.6 ppm) to control chicks also caused increased thymus weight compared to thymus weight from chicks fed either no selenium or organic selenium, but with infection, only organic selenium (0.3 or 0.6 ppm) maintained thymus weight comparable to weights of non-infected chicks. Gowdy and Edens (2003) also reported that the chicken T-cell-mediated wing web response to PHA-P in Sel-Plex®-fed broilers was less than the response in sodium selenite-fed birds. Presumably, the lesser delayed type hypersensitivity response associated with Sel-Plex® feeding was associated with increased GSH-Px activity in lymphocytes and granulocytes that migrate to the site of antigen stimulation (Brown et al., 2000).

Arthur et al. (2003) reported that selenium is essential in the activation of all elements of the immune system. The thymus seems to have a special

### Table 7. Mean serum thyroxin (T4, ng/mL), triiodothyronine (T3, ng/mL), and percentage levels in Sel-Plex®-fed, Sel-Plex® + selenite-fed and no supplemental selenium compared with the control (selenite) over a six week growing period.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>T4</th>
<th>T3</th>
<th>T4</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ng/mL)</td>
<td>(%)</td>
<td>(ng/mL)</td>
<td>(%)</td>
</tr>
<tr>
<td>No Se</td>
<td>6.05±c</td>
<td>144.3</td>
<td>0.47±c</td>
<td>72.0</td>
</tr>
<tr>
<td>Selenite</td>
<td>4.29±a</td>
<td>100.0</td>
<td>0.64±b</td>
<td>100.0</td>
</tr>
<tr>
<td>Sel-Plex®</td>
<td>3.99±b</td>
<td>88.1</td>
<td>0.77±a</td>
<td>120.0</td>
</tr>
<tr>
<td>Sel-Plex® + selenite</td>
<td>4.08±a</td>
<td>96.8</td>
<td>0.76±a</td>
<td>118.8</td>
</tr>
</tbody>
</table>

a,b,c In a column, means with unlike superscripts differ (P≤0.05).
affinity for selenium because it is the location for maturation of T cells that ultimately will control most aspects of the immune system. The thymus, at least in mammals, also contains selenium-dependent Type II deiodinase enzyme, which is used to locally convert T4 to T3 and rT3 to rT2. Thus, it is highly probable that any impairment of this enzyme could have effects on T cells and the immune system in diverse locations throughout the body of an animal. The thymus appears to be exquisitely sensitive to selenium as shown by the results of Gowdy and Edens (2003); and it appears that organic selenium is better tolerated than inorganic selenium in the thymus and possibly mature T cells.

Leng et al. (2003) compared the influence of either sodium selenite or organic selenium sources on immunity in layer chickens. They determined that organic selenium increased levels of tissue selenium more than 2-fold higher than with selenium derived from selenite. More importantly, Leng et al. (2003) were the first to show that the organic selenium caused an increase in CD3+, CD4+, and CD8+ surface markers on T cells located in several lymphoid structures in young chickens. The CD3+ marker is involved in signal transduction and is found in all T cells. The CD4+ marker is related to helper T cell-mediated expansion of the humoral immune response ascribed solely to B cells from the bursa of Fabricius, and the CD8+ T cell marker is associated with cytotoxicity and cell killing functions. Leng et al. (2003) concluded that organic selenium improved the status of the avian immune system by increasing the rate and ability of immunocompetent cells to respond to antigen challenge. They concluded that chickens fed organic selenium had more protection against potential pathogens than did chickens fed selenite. Chang et al. (1994) studied the influence of sodium selenite and vitamin E on T and B cell markers and lymphocyte proliferation. Lymphocyte proliferation was impaired by vitamin E and selenium deficiency, but the CD4+, CD8+ T cell populations increased slightly with vitamin E and selenium deficiency.

The relationship between the immune system and performance in poultry species is complex. It is generally believed that a heightened status of the active immune system is correlated with decreased performance. In fact, that situation can become established because an active immune system has higher energetic demands and nutrients are recompartmentalized to provide the energy required by the immunoactive cells. Increased demand from immunoactive cells decreases the nutrients that normally would be destined for protein production associated with growth. In animals, with an immune system conditioned to react quickly to an antigen challenge and clear that antigen more quickly, performance can actually be enhanced due to less time utilized in combating the numerous challenges poultry face every day in their specific environments. These activities might be enhanced in animals given organic selenium in Sel-Plex® as compared with animals fed inorganic sodium selenite. Evidence presented by Gowdy and Edens (2003) seem to support this hypothesis.

It is apparent that selenium-dependent 5′-deiodinase activity in poultry has a very important role to play. On the most fundamental basis, embryonic growth, livability and hatchability are dependent on availability of selenium from the dam, and provision of organic selenium to the dam improves all of these parameters for the embryo (Edens, 2002). Hatchability of chicken and turkey embryos is under the influence of thyroid hormones (Christensen and Biellier, 1982; Christensen, 1985). Christensen et al. (2002) determined that delayed conversion of T4 to T3 was associated with increased embryonic mortality and delayed hatchability. However, if T3 is elevated prior to certain critical periods, embryonic livability and hatchability can also be decreased (Christensen et al., 2002). Van der Geyten et al. (1997) have reported that deiodinase 1 (D1) and deiodinase 3 (D3) in chicken embryos develop in parallel to the plasma T3 profiles. D1 increased from day 14 of incubation to hatching. D3 increased from day 14 of incubation until day 17 of incubation then decreased. These enzymatic activities would indicate that there is maximized plasma T3 concentrations at the time of internal pipping and conversion to lung breathing by the embryo. More recently, an unpublished report from the poultry industry indicated that hatchability of turkey eggs from Sel-Plex®-fed hens had a higher hatch rate if they had to be stored for as long as 10 to 14 days.

As pointed out by Edens (1996; 2001), broiler performance is improved with the use of organic selenium. Much of the explanation for the improved performance has focused on improved antioxidant status in chicks and poults from organic selenium-fed hens. Furthermore, post-slaughter yield is improved with the use of organic selenium, and water retention as indicated by less drip loss is improved. Because it appears that T3 is very intimately involved in these responses, one must conclude that selenium
as organic selenium has a special role to play in the regulation of poultry selenium-dependent monodeiodinases that convert T₄ to T₃ and rT₃ to T₂.

**Thioredoxin reductase in chickens**

Thioredoxin reductase (TrxR) belongs to a superfamily of flavoenzyme disulfide oxidoreductases including glutathione reductase, mercuric ion reductase, dihydrolipoamide reductase, and alkyl hydroperoxide reductase (Williams, 1992). This selenium dependent enzyme has been reported to show increased activity after selenium supplementation to animals as compared with enzyme activity in selenium deficient animals (Hill et al., 1997; Berggren et al., 1999). However, Ganther and Ip (2001) have reported that monomethylated selenium in vivo did not increase TrxR activity and that high concentrations of selenium in a cell-free system inhibited TrxR. Wu et al. (2003) reported that there was an inverse relationship between dietary selenium and the activity and expression of TrxR in rat aorta. Wu et al. (2003) suggested that the mechanisms regulating transcription of GSH-Px and TrxR in the aorta are different. Wu and colleagues suggested that GSH-Px expression and activity are directly related to selenium intake, but TrxR activity is mediated mostly by generation of ROM. In long-term selenium deficiency studies by Wu et al. (2003), TrxR activity actually increased, selenium repletion of the rats caused a decrease in TrxR activity, and selenium adequate animals had the lowest activity of TrxR. The decreased activity of TrxR in the aorta of selenium-repleted animals might be a tissue specific response, because in other organs/tissues selenium deficiency caused a decrease in TrxR and GSH-Px activities (Berggren et al., 1999). Berggren et al. (1999) stated that TrxR behaved in a manner very different from other selenoenzymes in that it increased its activity with excess sodium selenite and that the increased activity was transitory eventually returning to lower activities even with continued high dosing of selenium. Therefore, these observations might indicate that TrxR is induced as a safety precaution when its selenocysteine is affected by severe oxidative stress (Gladshef et al., 1999). In mammalian fast growing transformed tumor cells with high generation of ROM, thioredoxin and TrxR activities are much higher than in normal cells (Liu and Stadtman, 1997; Gladshef et al., 1998; Ganther, 1999). There is an inverse relationship between TrxR and GSH-Px activity in tumor cells as compared with normal tissues or cell lines (Gladshef et al., 1998).

Thioredoxin reductase functions to stabilize disulfide bonds (-S-S-), free sulfhydryl groups (-SH), and to reduce thioredoxin. In the extracellular environment, TrxR interacts with disulfide bonds on cell surfaces and extracellular proteins. In the cytosol of the cell, TrxR primarily stabilizes the sulfhydryl groups and reduces thioredoxin. Thioredoxin is required inside the cell to provide reducing equivalents for many different substrates, primarily proteins that are vital to the survival of the cell. When thioredoxin undergoes oxidation, it is able to transfer reducing power to cellular proteins through TrxR, and TrxR, using electrons from NADPH, then reduces thioredoxin. All three mammalian TrxRs are selenium-dependent flavoproteins (Tamura and Stadtman, 1996).

Thioredoxin, the primary substrate for thioredoxin reductase, is expressed differentially in chickens as a product of a single copy gene and is similar to thioredoxins in other species (Jones and Luk, 1988). Thioredoxin is a high capacity electron donor for reductive enzymes that include ribonucleotide reductase, thioredoxin peroxidase, and through thiol/disulfide exchange it reduces cysteine residues in transcription factors to increase their binding to DNA thereby influencing gene transcription (Mustacich and Powis, 2000; Powis and Montfort, 2001). Thioredoxin also functions as a cell growth factor and inhibits apoptosis. Arner and Holmgren (2000) report that thioredoxin reduces hydroperoxides, ascorbate and selenite. Ascorbate is the biochemical link between vitamin E and selenium because it has been shown to recycle tocopheroxyl to tocopherol in vitro (Burk and Hill, 1999). The thioredoxin-thioredoxin reductase system, which maintains free sulfhydryls, and the GSH/GSH-Px system, which is a primary antioxidant system, work together to regulate a low intracellular redox potential (Arner and Holmgren, 2000).

The TrxR work in chickens has just begun, and preliminary observations suggest that chicken TrxR may be different from mammalian TrxR (Gowdy and Edens, unpublished). Some of our preliminary observations on TrxR are presented in Tables 8-10, representing the first observations on tissue and cellular distributions in chicken and the influence of different selenium sources on its activity in various tissues.

Organ distribution and activity of TrxR in 3-week old broiler cockerels from our current studies are presented in Table 8. Similar to the observations made by Berggren et al. (1999) with the rat, we observed that broiler chickens which were not provided
supplemental selenium in their diets had significantly lower TrxR activities than broilers given either sodium selenite or Sel-Plex®. A second observation in this distribution study was that the selenium source was important in regulating TrxR activity. With few exceptions, TrxR activity was higher in Sel-Plex®-fed cockerels than in sodium selenite-fed or combination (selenium + Sel-Plex®)-fed birds. In comparison to mammals, chickens have low TrxR activities (Smith and Levander, 2001), and the low activities found in these chickens reflect the fact that TrxR levels in normal tissues are very low (Liu and Stadtman, 1997).

The subcellular distribution of TrxR in chicken hepatic cells is presented in Table 9. The relatively high TrxR activity in a liver homogenate (primarily cytoplasmic) indicates the importance of the cellular content of the enzyme. When different fractions of the cell were analyzed for TrxR activity, the nuclear pellet and the mitochondrial lysate had the highest activities followed by post-mitochondrial supernatant, mitochondrial membranes, post-nuclear supernatant, and the mitochondrial pellet. Rigobello et al. (1998) reported that total TrxR activity of the mitochondrial pellet was higher than the mitochondrial matrix (lysate) in rat liver cells, but when specific activity was determined, the matrix TrxR activity was higher than that in the mitochondrial pellet, similar to the observation made in this investigation with chickens (Table 9). The post-nuclear and post-mitochondrial supernatant TrxR activities were comparable to the cytosolic activity because those post-supernatants are comprised of the cytosol. The observations with subcellular distribution of TrxR in chickens are similar to observations made in rat (Rozell et al., 1985;1988) and human tissue (Ejima et al., 1999; Chen et al., 2002). Rozell et al. (1985;1988) concluded that the distribution of thioredoxin and TrxR to subcellular structures such as the endoplasmic reticulum, secretory granules, plasma membrane, and at the subplasma membrane was consistent with the functions in protein processing, secretion, and formation of nascent protein disulfides. Chen et al. (2002) found high concentrations of TrxR in the mitochondria, lysosome, microsome and cytosol in human liver, and Ejima et al. (1999) found high TrxR activity (90%) in the cytosol of the placenta cells and about 10% of the total cellular activity in mitochondria. An important deduction from these observations is that TrxR plays a significant role in the nucleus and in the mitochondria. The role of TrxR in the nucleus should be apparent when it is understood that thioredoxin is used to enhance transcription of DNA (Mustacich and Powis, 2000; Powis and Montfort, 2001).

Table 8. Thioredoxin reductase activity in chicken tissues from 3-week-old male broilers fed supplemental levels of selenite, Sel-Plex®, a combination of 0.15 ppm selenite + 0.15 ppm Sel-Plex®, or no supplemental selenium 1.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control (0 Se)</th>
<th>Selenite (0.3 ppm)</th>
<th>Sel-Plex® (0.3 ppm)</th>
<th>Sel-Plex® + selenite (0.3 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>0.023a</td>
<td>0.084a</td>
<td>0.071a</td>
<td>0.053a</td>
</tr>
<tr>
<td>Lang</td>
<td>0.047b</td>
<td>0.082b</td>
<td>0.085b</td>
<td>0.034b</td>
</tr>
<tr>
<td>Heart</td>
<td>0.024b</td>
<td>0.062b</td>
<td>0.114b</td>
<td>0.056b</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.061a</td>
<td>0.087b</td>
<td>0.079a</td>
<td>0.051a</td>
</tr>
<tr>
<td>Brain</td>
<td>0.060a</td>
<td>0.076a</td>
<td>0.149b</td>
<td>0.129b</td>
</tr>
<tr>
<td>Breast muscle</td>
<td>0.028a</td>
<td>0.069ab</td>
<td>0.083a</td>
<td>0.066ab</td>
</tr>
<tr>
<td>Bursa</td>
<td>0.026a</td>
<td>0.070ab</td>
<td>0.098b</td>
<td>0.058ab</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.038a</td>
<td>0.092b</td>
<td>0.140a</td>
<td>0.076a</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.025a</td>
<td>0.045ab</td>
<td>0.065a</td>
<td>0.076a</td>
</tr>
<tr>
<td>RBC</td>
<td>0.030a</td>
<td>0.066a</td>
<td>0.040b</td>
<td>0.021a</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.034a</td>
<td>0.031a</td>
<td>0.025a</td>
<td>0.039a</td>
</tr>
</tbody>
</table>

1TrxR activity (at 412 nm) was determined using the DTNB assay that measures the NADPH-dependent reduction of the disulfide bond in 5,5'-dithiobis(2-nitrobenzoic acid). Calculated results are based on yield of 2 moles of 2-nitro-5-thiobenzoate per mole of NADPH consumed. Results are given as the µmol of NADPH oxidized per minute per mg of total protein.

Table 9. Subcellular distribution of chicken thioredoxin reductase activity (µmol NADPH/min/mg total protein) in liver cells.

<table>
<thead>
<tr>
<th>Cellular distribution</th>
<th>µmol/min/mg total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver homogenate</td>
<td>0.1072</td>
</tr>
<tr>
<td>Nuclear pellet</td>
<td>0.1289</td>
</tr>
<tr>
<td>Post-nuclear supernatant</td>
<td>0.0865</td>
</tr>
<tr>
<td>Mitochondrial pellet</td>
<td>0.0650</td>
</tr>
<tr>
<td>Post-mitochondrial supernatant</td>
<td>0.1066</td>
</tr>
<tr>
<td>Mitochondrial lysate</td>
<td>0.1333</td>
</tr>
<tr>
<td>Mitochondrial membranes</td>
<td>0.0930</td>
</tr>
</tbody>
</table>

TrxR activity (at 412 nm) was determined using the DTNB assay that measures the NADPH-dependent reduction of the disulfide bond in 5,5'-dithiobis(2-nitrobenzoic acid). Results are given as the µmol of NADPH oxidized per minute per mg of total protein.

We also wanted to determine the influence of feeding high levels of sodium selenite and Sel-Plex® on activity of TrxR in chicken liver homogenates (Table 10). This was part of a larger study in which the toxicity of Sel-Plex® was being evaluated in chickens ( Gowdy and Edens, 2003). In that study, we determined that as little as 1.2 ppm of sodium selenite...
was sufficient to allow signs of toxicity to develop in high-yield broiler chickens. At 5 ppm sodium selenite caused severe growth depression, and at 15 ppm all of the chickens fed sodium selenite had experienced severe growth depression and nearly all had died by two weeks of age. Contrary to those observations, there was no treatment-associated mortality in the Sel-Plex®-fed groups and body weights were comparable to controls fed 0.3 ppm Se. At two weeks of age, the chickens were killed by carbon dioxide asphyxiation and liver samples were collected from each bird and analyzed for TrxR (Table 10). It was interesting to observe that chickens given no supplemental selenium had the lowest liver TrxR activity. Feeding either sodium selenite or Sel-Plex® from 0.3 ppm to 15 ppm induced higher activities of TrxR, and Sel-Plex® at 15 ppm Se induced the highest activity of TrxR. This observation was similar to that of Berggren et al. (1999) who also reported selenium (as selenite) to be capable of inducing in vivo higher TrxR activity in rats. Thus, chickens and rats respond comparably with selenium-mediated induction of TrxR.

Table 10. Thioredoxin reductase activity (µmol NADPH/min/mg total protein) from chicken liver homogenates from 3 week old male broilers fed high levels of selenite or Sel-Plex®.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>µmol/min/mg total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0 ppm Se added)</td>
<td>0.042&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Selenite, 0.3 ppm Se</td>
<td>0.084&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sel-Plex®, 0.3 ppm Se</td>
<td>0.071&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Selenite, 5 ppm Se</td>
<td>0.081&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sel-Plex®, 5 ppm Se</td>
<td>0.098&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Selenite, 10 ppm Se</td>
<td>0.090&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sel-Plex®, 10 ppm Se</td>
<td>0.096&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Selenite, 15 ppm Se</td>
<td>0.102&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sel-Plex®, 15 ppm Se</td>
<td>0.146&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1TrxR activity (at 412 nm) was determined using the DTNB assay that measures the NADPH-dependent reduction of the disulfide bond in 5,5’-dithiobis(2-nitrobenzoic acid). Calculated results are based on yield of 2 moles of 2-nitro-5-thiobenzoate per mol of NADPH consumed. Results are given as the µmol of NADPH oxidized per minute per mg of total protein.</sup>

<sup>a,b,cMeans with unlike superscripts differ significantly (P ≤ 0.05).</sup>

The importance of thioredoxin reductase in living systems, such as the growing chicken, continues to be revealed. Currently, it is believed that TrxR functions to reduce thioredoxin and maintain a readily available supply of the reduced thioredoxin, which has numerous functions associated with growth and development. Second, TrxR functions to maintain cytosolic protein sulhydryls and disulfide bonds in a reduced state. Third, TrxR has antioxidant properties that come into play when the redox potential becomes more negative due to rapid and large production of ROM in cells. In that capacity, TrxR acts in a manner similar to GSH-Px as an antioxidant against ROM. Without these general functions, animals such as chickens and other food animal species would suffer from oxidative stress and become less productive.

**Summary**

- Selenium is essential in all animals.
- Organic selenium is the preferred form and has higher bioavailability than inorganic selenium.
- Sodium selenite is a pro-oxidant but selenomethionine is not.
- Feed supplements providing organic selenium are not the same; exercise caution in making selection.
- Selenium is unique among all trace minerals, requiring its own mRNA and SECIS.
- Selenomethionine in body protein provides reversible storage of selenium in tissues and organs.
- Selenomethionine is integrated into body protein, but inorganic selenium is loosely associated with thiols via a labile bond.
- Selenium must function through specific selenoproteins (25 known) encoded by at least 18 genes.
- Poultry express selenoproteins, but all avian selenoproteins are not yet described.
- Poultry are known to express selenium-dependent GSH-Px, iododeiodinase, and TrxR.
- Performance as indicated by body weight, livability, feed conversion, meat yield, and meat quality in poultry is influenced significantly by selenoproteins.
- Physiological functions as diverse as body temperature regulation, immunity, resistance to oxidative stress, reproduction, thyroid function, growth, development, all endocrine functions, digestive processes associated with pancreatic function, and more are influenced by selenium status in poultry.
References


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Hill, K.E., G.W. McCollum, M.E. Boeglin and R.F.


Alternatives to antibiotics in poultry production: responses, practical experience and recommendations

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During the past 50 years, the livestock and poultry industries have developed in several areas, including nutrition, genetics, engineering, management, and communications to maximize the efficiency of growth performance and meat yield. Now these industries must focus more attention on how animal agriculture affects the environment and food safety. As in many other industries, the global paradigm is shifting from an emphasis on productive efficiency to one of public security. Nothing demonstrates this paradigm shift more clearly than the issues concerning the use of antibiotic growth promoters. For the past four decades, antibiotics have been used in animal agriculture to improve growth performance and protect animals from the adverse effects of pathogenic and non-pathogenic enteric microorganisms. Now, antibiotics have come under increasing scrutiny because of the potential development of antibiotic-resistant human pathogenic bacteria after long use (Phillips, 1999; Ratcliff, 2000). In response to this apparent ‘threat’, the European Union banned the use of subtherapeutic levels of antibiotics to prevent disease or promote growth, starting with a ban on avoparcin in 1997 and a ban on virginiamycin, bacitracin, spiramycin, and tylosin in 1999. Antimicrobials scheduled to be banned by 2006 include avilamycin, bambermycin, salinomycin, and monensin. In June of 2003, McDonald’s Corp. announced that it would prohibit their direct suppliers from using antibiotics that are important in human medicine as growth promoters in food animals after 2004, and they created a purchasing preference for companies that work to minimize antibiotic use. Although banning antibiotic growth promoters may not be scientifically justified, the tide of public opinion is forcing animal agriculture to develop alternatives, or at least substantially reduce the amount of antibiotics used to maintain production efficiency and produce safe meat and egg products. Some of these alternatives may include significant changes in husbandry practices or the strategic use of enteric microflora conditioners, including acidifiers, probiotics, enzymes, herbal products, microflora enhancers, and immunomodulators. The objective of this paper is to briefly review the use of antibiotic growth promoters as enteric conditioners and discuss the potential of non-pharmaceutical alternatives.

Benefits of feeding antibiotics

Antibiotic usage in animal feeds has many benefits. It improves food safety by increasing animal health and reducing or eliminating certain pathogens. It reduces animal production costs and economic benefits are distributed along the food chain, including the feed industry, production animal agriculture, food processors, retailers, and consumers. Most of the cost savings attributed to antibiotics is from improved feed conversion, and this response is highest in fast-growing genetically improved animals reared in intensive production systems. Other cost savings come from faster growth rate, reduced mortality, greater resistance to disease challenge, improved reproductive performance, improved pigmentation, and better manure and litter quality. Rosen (1995) concluded from his review of 12,153 feeding studies that antibiotic growth promoters gave a positive response 72% of the time. The magnitude of responses was dependent upon the type of animal management, disinfection procedures, age of the farm buildings, and quality of the feed. Finally, the use of antibiotic growth promoters has a positive impact on two important issues facing animal agriculture: animal welfare and environmental stewardship. Animal welfare is definitely improved in animals that are
healthier due to the disease-suppressing effects of antibiotics. The improved utilization of dietary nutrients by supplemental antibiotics results in significant reduction in nitrogen, phosphorus, and other nutrients excreted into the environment (Cromwell, 1999).

**Antibiotic modes of action**

Antibiotics are natural metabolites of fungi that inhibit the growth of bacteria. They function by altering certain properties of bacterial cellular metabolism resulting in impaired growth or death. Some antibiotics interfere with the building and maintenance of the cell wall, while others interrupt proper protein translation at the ribosomal level. Because of their elevated rate of growth and proliferation, bacteria are vulnerable to antibiotics that target active cellular metabolism. Limiting the growth and proliferation of certain bacteria and inhibiting the production of various toxins restricts the influence that the microbe has upon the host organism. This enables the host to grow and perform better than if grown under normal challenge conditions.

The term ‘Growth Promoter’ has been used for years to describe the use of subtherapeutic levels of antibiotics to improve growth performance. It is an inappropriate term to describe this use of antibiotics because they do not promote growth as do anabolic hormones, such as growth hormone or estrogen-like compounds. This may be why the general public confuses this term with the use of anabolic hormones. The poultry industry does not use anabolic hormones as do the swine and cattle industries. Instead of calling them ‘Growth Promoters’, they should be called ‘Growth Permitters’ because they allow the animal to express its genetic potential for growth without compromise.

Antibiotics limit the growth of detrimental microbes, such as *Clostridium perfringens* (Truscott and Al-Sheikhly, 1977). They also limit the growth and colonization of numerous non-pathogenic species of bacteria in the gut, including lactobacilli, bifidobacteria, bacteroides, and enterococci (Tannock, 1997). Antibiotics reduce the production of antagonistic microbial metabolites, such as ammonia (Zimber and Visek, 1972), which adversely affect the physiology of the host animal. Subtherapeutic levels of antibiotics in the diet also reduce weight and length of the intestines (Visek, 1978; Postma et al., 1999). A thinner intestinal epithelium in antibiotic-fed animals may enhance nutrient absorption (Visek, 1978) and reduce the metabolic demands of the gastrointestinal system. The minimization of gastrointestinal bacteria may also ease the competition for vital nutrients between the bird and the microbes (Ferket, 1991). Finally, antibiotics may reduce the adverse effects of immunological stress on growth performance by lowering the enteric microbial load. Over-stimulation of the host immune system by the resident microflora could impair the optimum growth and performance of the bird (Cook, 2000; Klasing, 1988).

**The antibiotic resistance debate**

During the last 10 years, the use of growth promoting antibiotics has been criticized for their possible role in the occurrence of antibiotic-resistant microbes. Numerous reports have been issued concerning the effects of agriculture-related antibiotics on the emergence of antibiotic resistance in human pathogens (SCAN Report, 1999; DANMAP, 2000). Although a complete ban on the use of subtherapeutic doses of antibiotics in animal feed has not yet been enforced in many countries, this day may eventually come. There is some evidence that the use of antibiotic growth promoters in animal and poultry feeds is associated with bacterial resistance in human disease therapy. Rapid selection for resistant bacteria when subtherapeutic levels of antibiotics are fed occurs because of the plethora of bacteria in the gut of animals, the high mutation rates among these bacteria, and the frequent transfer of genes including resistance genes. Mathew et al. (2002) demonstrated that selection for resistant bacteria can occur in as little as two days following administration of a feed-based antibiotic. Wide use of antibiotic growth promoters in poultry is one reason the public is placing some blame for antibiotic resistance of potential pathogens on the poultry industry. Gustafson and Bowen (1997) reported that antibiotic resistance of indigenous *E. coli* of poultry has remained at a relatively high level since the 1950s. However, Lou et al. (1995) reported that removing antibiotics from a swine herd for now over 30 years has not eliminated antibiotic resistance. So, the question that needs to be answered is: Can a ban on the use of AGPs reverse the trend in increasing antibiotic resistance of human pathogens? This question can be answered from recent experiences after the European ban on AGPs.

Following the ban of all food animal growth promoting antibiotics by Sweden in 1986, the
European Union banned avoparcin in 1997, and bacitracin, spiramycin, tylosin, and virginiamycin in 1999. As a consequence of public pressure following the ban in 1999, the use of the two remaining antibiotics, bambermycin and avilamycin is scheduled to be banned in 2006. This ban is logical if the antibiotics banned from prophylactic usage were commonly used in human medicine, but that is not the case. Only bacitracin is routinely used in human medicine. In order to protect their position in the European market, the Danish government instituted a voluntary ban on the use of AGPs along with a penalty tax for use in 1998. In effect, Denmark has become an ideal laboratory to test the consequences of a total European Ban on AGPs. By 2000, the complete ban on the use of AGPs was in effect in Denmark, but then enteric disease problems and mortality rates began to mount and the therapeutic use of antibiotics began to rise sharply. By 2001, the total consumption of therapeutic antibiotics almost reached the same amount as the total consumption of AGPs before the ban was instituted. In effect, AGPs (avilamycin, virginiamycin, bacitracin, and tylocin) that are not typically used to treat human disease were replaced by therapeutic antibiotics (ampicillin, erythromycin, streptomycin, tetracycline, etc) that are used to treat human disease pathogens (Hayes and Jensen, 2003). For example, tetracycline use in Denmark increased from 12,100 kg in 1998 to 27,000 kg in 2001. Now Denmark has mounting tetracycline resistance in human pathogens, such as *Salmonella typhimurium* and *Campylobacter jejuni* (DANMAP, 2001). Isn’t it ironic that the policy against the use of AGPs actually resulted in an increase in resistance to antibiotics that the public is most concerned about? A simple ban on AGPs will not solve the antibiotic resistance problem, but may lead to greater risks to human and animal health (Casewell et al., 2003). Therefore we need to strategically use different feed additives and management practices that will minimize the use of both AGPs and therapeutic antibiotics.

**General strategies to control gut health without antibiotics**

Effective use of feed additives to manage gut health is dependent upon some degree of understanding of their mechanisms of action. Clearly, the modes of action of growth promoting antibiotics and their alternatives can differ considerably. Subtherapeutic antibiotics work in part by decreasing the microbial load in the gut, resulting in a reduction in energy and protein required to maintain and nourish the intestinal tissues. Because energy required to maintain the gut accounts for about 25% of the total basal metabolic needs of an animal (Croom et al., 2000), any reduction in gut tissue mass can have a significant impact on the amount of energy available for growth and caloric conversion efficiency. The reduced microbial load in the gut by subtherapeutic levels of antibiotics also reduces immunological stress, resulting in more nutrients partitioned toward growth and production rather than toward mechanisms of disease resistance. In contrast, most alternative compounds do not reduce overall microbial loads in the gut and thus will not promote growth by a mechanism similar to antibiotics. Instead, they alter the gut microflora profile by limiting the colonization of unfavorable bacteria while promoting the fermentation of more favorable species. Consequently, alternatives to antibiotics promote gut health by several possible mechanisms including: altering gut pH, maintaining protective gut mucins, selection for beneficial intestinal organisms or against pathogens, enhancing fermentation acids, enhancing nutrient uptake, and increasing the humoral immune response. Strategic use of these alternative compounds will help optimize growth provided they are used in a manner that complements their modes of action.

**SANITATION AND PATHOGEN LOAD REDUCTION**

There is considerable evidence that subtherapeutic antibiotics or alternative compounds are most effective when fed to animals raised in unsanitary environmental conditions. Good barn sanitation, pest control, biosecurity practices, and litter or manure management are necessary to reduce pathogen load and exposure and minimize the need for antimicrobial therapy. Water must be clean and drinkers must be properly maintained to minimize spillage and prevent a bloom of pathogens in the litter and environment of the animals. Implementation of a good sanitation program is usually much less costly than any disease treatment.

**ENHANCE PATHOGEN COLONIZATION RESISTANCE**

Colonization of enteric pathogens is dependent upon the degree of resistance afforded by the stability of
the resident microflora and the integrity of the intestinal mucin barrier in the animal. Older animals are much less susceptible to the colonization of enteric pathogens than young animals because they have a more stable and diverse gut microflora that competitively excludes pathogen colonization. In contrast, the ability of pathogens to colonize in the gut increases after antibiotic administration because of a loss of resident microflora. The stability of resident microflora can be enhanced by the administration of competitive exclusion cultures (probiotics) or feeding prebiotic compounds that feed the beneficial microflora. Hollister et al. (1999) reduced salmonella colonization in chicks by feeding a live cecal culture from salmonella-free poultry. Fedorka-Cray et al. (1999) have shown similar response to microbial cultures in young swine. Gram-positive bacteria, including Lactobacillus, Enterococcus, Pediococcus, Bacillus, and bifidobacteria, and fungi of the Saccharomyces (yeast) genus are often fed after antibiotic therapy as a means of re-introducing a beneficial flora to the gut of affected animals. Beneficial bacteria inhibit the colonization of pathogens by producing volatile fatty acids that reduce the pH of the brush-border microenvironment or they can block the attachment of pathogens. Organic acids have strong antibacterial effects, especially to Gram-negative pathogens. Blomberg et al. (1993a) also demonstrated that undefined compounds in a culture of lactobacilli inhibit the attachment to intestinal components of pigs by pathogenic K88 E. coli. They suggested that compounds produced by the lactobacilli or the lactobacilli themselves bound to the receptor of K88 E. coli in pig intestine, thereby preventing the colonization by the E. coli.

Mucins and glycoproteins associated with the intestinal brush border serve as a very important barrier protecting the delicate absorptive surface from the abrasive action of feedstuffs, bacteria colonization, and toxins. Mucin, produced by goblet cells, is secreted in response to the degree of insult on the absorptive surface of the gut. Glycoproteins of gut mucins specifically bind pathogens and reduce their colonization by serving as alternative binding sites to receptors on host enterocytes. For example, pathogenic E. coli K88 adhesins were found to bind to ileal mucus from pigs, and Blomberg et al. (1993b) concluded that the intestinal mucus might intercept these pathogens before they can attach to intestinal tissues and cause disease. Dietary factors that result in increased mucus secretion may thus indirectly enhance an animal’s ability to resist pathogen colonization. There is a complex balance between the gut ecosystem and intestinal mucins, and this balance can be altered by enteric health conditions and the diet. Although intestinal mucins and glycoproteins have a protective function, they also serve as a nutritional substrate for some bacteria that thrive in a galactose-rich environment, such as bifidobacteria (Roy et al., 1991). In pigs, Pestova et al. (2000) observed a significant decrease in intestinal mucins following weaning, and this was partially prevented by the inclusion of galactose in the post-weaning diet. Apparently, the lack of galactose in the post-weaning high starch diet increased the scavenging of galactosyl units in mucins by some microflora, thus promoting the degradation of the protective mucin barrier. Dietary inclusion of compounds that feed beneficial bacteria, such as bifidobacteria, should alleviate their attack on the protective mucins. Such compounds include oligosaccharides or enzymes that liberate galactose from galactosyl polymers, such as galactomannans. More research must be done in this area of interest.

**IMMUNE RESPONSE AUGMENTATION**

The immune system is the primary defense mechanism of the animal against infectious disease. Augmentation of humoral and cell-mediated immunity will increase an animal’s ability to resist disease. Although there is a small nutrient cost in the production of immunoglobulins, good antibody titer levels indicate a far more efficient capacity to resist disease by humoral immune responses than an active inflammatory response (Humphrey et al., 2002). A pro-inflammatory innate immune response is associated with the mobilization of nutrients away from growth and suppression of feed intake. Thus, dietary immunomodulators or vaccines that enhance humoral immunity and minimize immunological stress will affect growth performance most positively.

Although there is now a considerable amount of knowledge about systemic immunity, knowledge about gut-associated immunity is still primitive. The gut is a major interface where the immune system can sample the potential disease antigens in the animal’s environment and mount a defensive strategy to resist disease. Therefore, the resident microflora will have a marked effect on the amount and profile of immune factors, such as immunoglobulins. Perdigon et al. (1991) observed that specific lactobacilli fed to mice resulted in enhanced protection against S. typhimurium and E. coli by...
increasing IgA production. IgA, predominantly found in the mucus secretions in the respiratory tract and gut, function to attenuate antigens and present them to lymphocytes for degradation and stimulation of the production of specific antibodies. Dietary supplementation of mannan-oligosaccharide (BioMos®) has also been shown to enhance IgA titers in the plasma of poultry (Savage et al., 1996) and sow’s milk (O’Quinn et al., 2001).

An alternative to feeding dietary factors that stimulate gut-associated humoral immunity may be feeding specific antibodies that neutralize pathogenic organisms. To produce the specific antibodies, laying hens are exposed to particular antigens to stimulate the production of immunoglobulins, which are deposited in the egg. These immunoglobulins are then harvested from the eggs and fed to susceptible young animals. There may be some limitations to this technology, since these immunoproteins are sensitive to heat treatment during feed processing and the digestive process of the animal.

**Nutritional strategies and feed additives**

**DIET DIGESTIBILITY AND ENZYME SUPPLEMENTATION**

Gut health and enteric disease resistance is often dependent upon the digestibility of feed components and feed formulation. Poorly digested protein meals due to improper heat processing causes the proliferation of putrifying bacteria in the hindgut, which increases toxic metabolites (ammonia and biogenic amines) that compromise gut health. In agreement, antibiotics are most effective in birds fed diets containing high levels of indigestible protein (Smulders et al., 2000). Similarly, poultry feed diets containing high levels of poorly digested non-starch polysaccharides (NSP) from wheat, barley or rye are more susceptible to enteric disease, such as necrotic enteritis (Riddell and Kong, 1992; Kaldhusdal and Skjerve, 1996). Langhout (1999) observed that dietary NSP significantly increases gut populations of pathogenic bacteria at the expense of beneficial bacteria. However, the digestibility of wheat, barley, rye, triticale and even corn-based diets can be significantly improved through use of exogenous enzymes including xylanases, phytases and β-glucanases. The response to dietary enzyme supplementation is greater when antibiotics are not used than when they are, but the performance responses do not approach the level that is observed when diets contain enzymes and antibiotics together (Bedford, 2000; Elwinger and Teglof, 1991; Danicke et al., 1999). In a comprehensive literature review, Rosen (2001) concluded that the effect of enzymes was nearly equivalent to the effects of antibiotics on gain and FCR, and that in combination there was further improvement, but less than the sum of the two. Enzymes are perhaps the most extensively reviewed products that seem to be capable of limiting the performance losses associated with removal of antibiotic growth promoters.

Because supplemental enzymes mediate their beneficial effects primarily by enhancing feed digestibility and nutrient availability to the host, it must be assumed that they also influence the gut microbial ecosystem. The use of enzymes has been shown to alter the gut microflora populations in the small intestine and caeca (Chocet et al., 1996; Hock et al., 1997; Bedford, 2000) and reduce mortality rates (Rosen, 2001). Such benefits are brought about by a more rapid digestion and absorption of starch, protein and fat from the small intestine, which effectively limits available substrate for the resident flora. In general, the improvement in nutrient digestibility achieved for the host by the use of an appropriate enzyme is much smaller than the concomitant loss of substrate experienced by microflora resident in the large intestinal. This starch and protein removal effect is coupled with the production of exogenous enzyme for fiber-derived oligomers, which serve as substrate for specific populations of bacteria that seem to benefit the host (Bedford, 2000).

**ACIDIFIERS AND ORGANIC ACIDS**

Clostridia and pathogenic coliform bacteria often associated with enteric disease do not grow well in media of low pH, so any means to reduce gut pH should improve an animal’s resistance to enteric disease. Because organic acids have strong bacteriostatic effects, they have been used as salmonella-control agents in feed and water supplies for livestock and poultry. Organic acid blends have also been used as acidifiers in baby pig diets to reduce enteric disease, but the benefit for poultry seems to be less conclusive. Dietary acidifiers may work better in baby pig diets because they have more limited hydrochloric acid production than chicks. Moreover, dietary organic acids are easily neutralized in the duodenum unless they are delivered to the ileum and below by adsorbent vehicles.
HERBS, SPICES, AND ESSENTIAL OILS

Herbs, spices, and plant extracts have been used to make human foods more appetizing for centuries, and many of them are recognized for their health benefits. Some of these compounds stimulate appetite (e.g. menthol from peppermint), provide antioxidant protection (e.g. cinnamaldehyde from cinnamon), or suppress microbial growth (carvacrol from oregano). These plant-based antimicrobial compounds, which function in a fundamentally similar way to antibiotic compounds produced by fungi, could be used to replace some antibiotic growth promoters. To be most effective as growth promoters, these herbal antimicrobial compounds must be supplemented to the feed in a more concentrated form than found in their natural source. As with antibiotics, continued use of these plant-based antimicrobials may result in the development of resistance in some pathogenic bacteria. However, more research is necessary to confirm this risk.

Essential oils from oregano are showing the greatest potential as an alternative to antibiotic growth promoters. Oregano contains phenolic compounds, such as carvacrol, that have antimicrobial activity (Akagul and Kivanc, 1988). Like antibiotics, oregano essential oils modify the gut microflora and reduce microbial load by suppressing bacteria proliferation. There are some claims that oregano oil can replace anticoccidial compounds, not because they inactivate coccidia, but because they increase the turnover of the gut lining and prevent coccidial attack by maintaining a more healthy population of gut cells (Bruerton, 2002). This mode of action would increase the animal’s maintenance energy requirement because enterocyte turnover is a major proportion of the basal metabolic rate.

OLIGOSACCHARIDES

Oligosaccharides are promising alternatives to antibiotic growth promoters because they facilitate and support the symbiotic relationship between host and microflora. Fructooligosaccharide (FOS) and mannanoligosaccharide (MOS) are two classes of oligosaccharides that are beneficial to enteric health, but they do so by different means.

Fructooligosaccharide (FOS)

Fructooligosaccharide compounds are inulin-type oligosaccharides of D-fructose attached by β(2-1) linkages that are attached to a D-glucosyl residue at the end of the chain (Yun, 1996). A sucrose unit attached to one additional fructose residue is commonly referred to as 1-kestose. Nystose contains two additional fructose units, and three additional fructose units is designated as 1º- β-fructofuranosyl (Hidaka and Hirayama, 1991). Fructooligosaccharides are found in numerous plants such as the onion, Jerusalem artichoke, garlic, banana, chicory, asparagus, and wheat.

Fructooligosaccharides influence enteric microflora by ‘feeding the good bacteria’, which competitively excludes the colonization of pathogens. Dietary supplementation of FOS provides selective enrichment of lactobacilli (Mitsuoka et al., 1987) and bifidobacteria (Hidaka et al., 1991). Patterson et al. (1997) found that cecal bifidobacteria concentrations were increased 24-fold and lactobacilli populations increased 7-fold in young broilers fed the FOS-enriched diets. Fructooligosaccharides are well utilized by the majority of bifidobacteria strains (B. longum, brevis, and infantis) with the exception of B. bifidum (Hidaka and Hirayama, 1991). The bacteroides group also showed a tendency to utilize FOS as a growth source, while L. fermentum, E. coli, and C. perfringens failed to utilize FOS as a fermentative carbohydrate source. Bifidobacteria readily ferment FOS because of the innate secretion of a β-fructoside enzyme. Bifidobacteria may inhibit other microbes because of their acidic surroundings from the high production of VFAs or the secretion of bacteriocin-like peptides. The improvement in gut health conditions by dietary FOS supplementation often results in improved growth performance. Ammerman et al. (1988) demonstrated that the addition of either 0.25% or 0.50% dietary FOS improved feed efficiency from 1 to 46 days of age and reduced mortality when fed at the higher level (0.50%). FOS-treated birds also had less air sac lesions at day 46.

Mannanoligosaccharide (MOS)

Unlike FOS, MOS is not used as a substrate in microbial fermentation, but it still exerts a significant growth-promoting effect by enhancing the animal’s resistance to enteric pathogens. Bio-Mos® (Alltech Inc., Nicholasville, KY) is the commercial source of MOS that has been used in most of the published research literature. Based on the scientific literature, Bio-Mos® enhances resistance to enteric disease and promotes growth by the following means: 1) inhibits colonization of enteric pathogens by blocking bacterial adhesion to gut lining; 2) enhances
immunity; 3) modifies microflora fermentation to favor nutrient availability for the host; 4) enhances the brush border mucin barrier; 5) reduces enterocyte turnover rate; and 6) enhances the integrity of the gut lining.

Inhibition of pathogen colonization

Mannan oligosaccharides, derived from mannans on yeast cell surfaces, act as high affinity ligands, offering a competitive binding site for a certain class of bacteria (Ofek et al., 1977). Gram-negative pathogens with the mannose-specific Type-1 fimbriae attach to the MOS instead of attaching to intestinal epithelial cells and they move through the gut without colonization. Dietary MOS in the intestinal tract removes pathogenic bacteria that could attach to the intestinal epithelium (Newman, 1994). Mannose was shown by Oyofo et al. (1989a) to inhibit the in vitro attachment of S. typhimurium to intestinal cells of the day-old chicken. Then Oyofo et al. (1989b) provided evidence that dietary D-mannose was successful at inhibiting the intestinal colonization of S. typhimurium in broilers. The ability of MOS to interfere with the attachment of pathogenic bacteria in the gut raises the possibility that it could also inhibit the binding between bacteria that is required for plasmid transfer via conjugation. This kind of inhibition of plasmid transfer in the digestive tract of mice colonized with human microflora has been described using lactose (Duval-Iflah, 2001). Lou (1995) demonstrated that dietary Bio-Mos® supplementation decreased the proportion of specific groups of Gram-negative antibiotic resistant fecal bacteria in swine.

In an effort to confirm that MOS inhibits pathogen colonization, Spring et al. (2000) screened different bacterial strains for their ability to agglutinate mannan oligosaccharides in yeast cell preparations. Five of seven strains of E. coli and 7 of 10 strains of S. typhimurium and S. enteritidis were agglutinated by Bio-Mos® and Sac. cerevisiae cells. However, strains of S. choleraesuis, S. pullorum, and Campylobacter were not agglutinated. Although Bio-Mos® does not bind clostridia, it does reduce clostridial numbers in some trials, possibly by enhancing the mucin barrier or stimulating gut associated immunity.

Enhancement of immune function

Mannan oligosaccharide has been shown to have a positive influence on humoral immunity and immunoglobulin status. As mentioned above, a good humoral immune response is nutritionally a more efficient means to resist disease than an active inflammatory response (Humphrey et al., 2002). Savage et al. (1996) reported an increase in plasma IgG and bile IgA in pouls fed diets supplemented with 0.11% Bio-Mos®. An increase in antibody response to MOS is expected because of the ability of the immune system to react to foreign antigenic material of microbial origin. Portions of the cell wall structure of the yeast organism Saccharomyces contained in MOS has been shown to elicit powerful antigenic properties (Ballou, 1970). However, MOS may also enhance humoral immunity against specific pathogens by preventing their colonization leading to disease, yet allowing them to be presented to immune cells as attenuated antigens. Indeed as MOS facilitates the secretion of IgA into the gut mucosa layer, pathogenic agents become more labile to the phagocytic action of gut-associated lymphocytes.

All animals reared under commercial field conditions are subjected to immunological stress, depending on the pathogen load in the environment and the vaccination program. The release of cytokines associated with inflammation and the innate immune response results in fever (which reduces appetite), causes the mobilization of body reserves (glucose, amino acids, and minerals) away from liver, muscle and bone, suppresses nutrient absorption in the gut, and increases body fluid losses as diuresis and diarrhea. The positive growth performance effects observed among animals in studies with Bio-Mos® may be partly due to its effect on acute immunological stress. Although MOS may enhance humoral immunity, there is some evidence that it may suppress the pro-inflammatory immune response that is detrimental to growth and production. To test this hypothesis, Ferket (2002) induced an acute immune stress in 14-day old turkey pouls by intraperitoneal injection of LPS from S. typhimurium strain SL 684. The poult’s weights were increased. In other words, the Bio-Mos®-fed birds retained normal body temperature after exposure to a pro-inflammatory antigen, while the controls and virginiamycin-fed birds expressed elevated body temperature. Under commercial
conditions where birds are subjected to chronic immunological stress, Bio-Mos® may help reduce the pro-inflammatory response and associated depression in feed intake and growth.

**Effects on gut microflora fermentation and dietary energy utilization**

Even though the ceca are the primary site of gut microflora fermentation, microbial fermentation in the jejunum has a greater influence on digestion and nutrient absorption. Measurement of volatile fatty acid (VFA) content and pH of the jejunum digesta is one way to evaluate the influence of feed additives on microbial fermentation. In a study with turkeys, Ferket (2002) observed dietary supplementation of Bio-Mos® and antibiotics reduced total VFA content of jejunum digesta by about 40%. Most of this effect was attributed to a reduction in propionic acid, which is the major fermentation product of microflora that use starches and sugars as their primary substrates. Therefore, Bio-Mos® may improve dietary energy availability by reducing the microflora-host competition for available starches and sugars. Indeed, apparent metabolizable energy of the diet was increased by about 3% when Bio-Mos® or virginiamycin was supplemented to the diet. Another benefit to dietary inclusion of Bio-Mos® was a decrease in jejunum digesta pH and ammonia concentration in comparison to the antibiotic-fed birds. Lower gut pH suppresses the proliferation of putrifying bacteria that excrete ammonia as their fermentation by-product, and ammonia has a detrimental effect on the integrity of gut tissues.

**Effects on gut tissue integrity and health**

The beneficial effects of MOS on the gut microflora, nutrient utilization, and growth performance may be associated brush border morphology and how it influences enteric disease resistance. To test this hypothesis, Ferket (2002) conducted an experiment to ascertain effects of Bio-Mos® and virginiamycin on jejunum villi morphology. Commercial Hybrid® poultcs were fed a corn-soy control diet or diets supplemented with 1 kg Bio-Mos®/tonne or 20 g virginiamycin/tonne starting at 1 day of age. At 14 days of age, 8 birds per treatment pen were sampled for morphometric measurements including villus height, crypt depth, muscularis thickness, and goblet cell number.

Bio-Mos® had the greatest effect on villi morphology. Although villus height was unaffected by Bio-Mos®, a decrease in crypt depth approached significance and villi height:crypt depth ratio was significantly greater than the control or virginiamycin treatments. Iji *et al.* (2001) also observed an increase in jejunal villi height:crypt depth ratio by Bio-Mos® supplementation in broilers, but this was due to a significant increase in villi height rather than crypt depth. These researchers also observed that Bio-Mos® significantly increased protein/DNA of jejunal mucosa, and increased the brush border enzymes maltase, leucine aminopeptidase and alkaline phosphatase. Turkeys receiving Bio-Mos® in our experiment also exhibited a thinner muscularis layer and increased the number of goblet cells per mm of villus height as compared to control birds.

The mucus gel layer coating the surface of the intestinal epithelium is the first major barrier to enteric infection. Hence, the production of mucus, as indicated by the number of goblet cells, is an important feature in the protective scheme against pathogens. Feeding Bio-Mos® resulted in an increased proliferation of goblet cells into the surface of the villus membrane. The innate immune system recognizes key molecular structures of invading bacteria, including lipopolysaccharides, peptidoglycans, and possibly the mannose structures in the cell walls of yeasts. Oligosaccharides containing mannose have been shown to affect the immune system by stimulating liver secretion of mannose-binding protein. This protein, in turn, can bind to bacteria and trigger the complement cascade of the host immune system (Newman, 1994). Intestinal microbes might influence goblet cell dynamics by releasing bioactive compounds or indirect activation of the immune system (Bienenstock and Befus, 1980).

**Conclusion**

In response to consumer demands and government regulations, today’s intensive animal agriculture industry must adapt to producing animals in a world without antibiotic growth promoters. This paper presented several alternatives to antibiotics to manage gut health. Although no single alternative may be as effective as antibiotics, a combination of strategies and feed additives can be used to achieve good gut health and growth performance. The key to selecting the most cost effective approach will depend upon the production requirements of each company, and the type of production challenges they face.
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Facing the realities of poultry health and performance without antibiotics in Europe

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Introduction

Maintaining the structure of the digestive tract in good health is critical for successful rearing of broilers. Dietary factors disrupting mucosal integrity or motility of the gastrointestinal tract (GIT) might induce enteric disorders, wet litter problems, poor pigmentation and inefficient growth. Enteric disorders have been managed through dietary changes including the use of in-feed growth promoters and animal proteins. Addition of certain antibiotics to feed at low levels is a common practice in poultry production and has been shown to improve weight gain and feed efficiency in the range of 1 to 5% (Thomke and Elwinger, 1998a). The reasons for the improvement are not well understood but some intestinal organisms such as clostridia and other gram-positive germs are inhibited by these antibacterial agents. In fact, the restrictions imposed on the use of in-feed antibiotics and proteins of animal origin in many European countries have increased the incidence of clostridiosis and other enteric diseases; and the cost of broiler meat production has increased by around 0.01 €/kg (Mateos et al., 2001a; Van der Eijk, 2002). However, consumer concerns about recycling animal proteins and cross-resistance related to the use of additives requires new methods to protect enteric health and improve broiler performance.

Many poultry integrators in Europe are producing feeds without growth promoters, based exclusively on vegetable feedstuffs. Under these circumstances, problems related to necrotic enteritis (NE), feed passage, and overgrowth of intestinal microflora are frequently reported. Numerous natural products, including organic acids, probiotics, prebiotics, plant extracts, and immune stimulants have been proposed for the control of pathogens in the GIT (Flickinger, 2003; Hooge, 2003; Chaveeraach et al., 2004). Also, manipulation of the composition and nutrient content of the diet might help to improve GIT health. Options to minimize enteric diseases associated with microflora changes include the use of highly digestible feeds to improve the structure of the gut, adequate processing of raw materials and diets, and the use of exogenous enzymes, organic acids, yeasts, and other additives (Lilburn, 1998; Mateos et al., 2002). However, data supporting the effectiveness of these techniques are equivocal, and changes in flock management, early detection of symptoms of diseases, and careful design of the feeding program are required to reduce the incidence of enteric problems.

Post-hatch nutrition, feed management and chick productivity

Growth of broilers during the first days post-hatch is of paramount importance for ultimate performance of poultry reared for meat. During the first week of life allometric growth is maximal and the chick multiplies initial body weight 4 to 5-fold. In commercial operations a large proportion of chicks remain without feed for more than 36 hrs after removal from the incubator as placement on the farm is frequently delayed because of hatchery processing and transportation. Furthermore, the yolk sac at hatch contains less than 1 g of triglycerides and is almost absent by the third to fourth day of life (Murakami et al., 1988; Bigot et al., 2003). Therefore, residual triglycerides in the yolk sac are not a good reservoir of nutrients and early access to feed is critical for the newborn chick (Lilburn, 1998). Early access to feed and water stimulates the growth of the GIT and its
absorptive capacity and improves gut integrity and subsequent performance (Moran, 1990; Noy and Sklan, 1999; Corless and Sell, 1999). The GIT adapts to the nature of the digestive contents, a response that is modulated by the health status of the gut. In case of disturbances, physiological responses take place with overgrowth of pathogenic bacteria and a reduction of the appetite. In practical conditions birds that eat more during the first week of life achieve the best final weights and feed conversion at slaughter (Martins, 2003).

The nutrient requirements of baby chicks and poults after hatching are not precisely known. Lilburn (1998) proposes feeding highly digestible protein ingredients in combination with corn for the first 10 days of life to meet the energy and protein needs of young birds. Batal and Parsons (2002a) observed that the ME(n) value for chicks of corn diets based on soybean or rapeseed meal was very low at 2 and 4 days of age, but increased afterwards. However, the ME(n) of a dextrose-casein diet was high at 2 days and no further improvements were observed with age (Table 1). Sulistiyanto et al. (1999) also indicated that casein was better utilized than fish meal and soybean meal for chicks less than 10 days of age. They also found that energy from corn was utilized better than energy from wheat and sorghum but not better than energy from different fat sources. On the other hand, Noy and Sklan (2002) observed that baby chicks do not respond to high-fat starter diets; and Sklan (2003) indicated that lipoprotein production might limit fat use in very young broilers.

Table 1. Influence of age on the ME(n):GE ratio of diets for chicks (%).1,2

<table>
<thead>
<tr>
<th>Age, (days)</th>
<th>Corn-soybean meal</th>
<th>Corn-canola meal</th>
<th>Corn-synthetic amino acids</th>
<th>Dextrose-casein</th>
<th>Pooled SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>6667</td>
<td>6334</td>
<td>8444</td>
<td>8865</td>
<td>0.8</td>
</tr>
<tr>
<td>3-4</td>
<td>6867</td>
<td>6434</td>
<td>8444</td>
<td>8865</td>
<td>0.6</td>
</tr>
<tr>
<td>7</td>
<td>7067</td>
<td>6534</td>
<td>8774</td>
<td>8865</td>
<td>0.4</td>
</tr>
<tr>
<td>14</td>
<td>7367</td>
<td>6854</td>
<td>8984</td>
<td>8865</td>
<td>0.2</td>
</tr>
<tr>
<td>21</td>
<td>7367</td>
<td>6954</td>
<td>8984</td>
<td>8965</td>
<td>0.4</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>0.6</td>
<td>0.7</td>
<td>1.0</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

1Batal and Parsons (2002a).
2Soy oil was used as the main fat source of the diets.
*Means within a column differ significantly (P<0.05).
**Means within a row differ significantly (P<0.05).

Digestibility of nutrients

The National Research Council (1994) assumes that digestibility of nutrients is independent of age, an assumption that is no longer accepted (Batal and Parsons, 2002a; Mateos et al., 2002). Digestion and absorption of nutrients early in life depends primarily on pancreatic enzyme activity (Nitsan et al., 1991a, b), but the pancreas is immature at hatch. As a consequence, dietary nutrients are poorly utilized during the first 10 days post-hatching. Gracia et al. (2003a) have reported that in the broiler chick, the maximal weight (g organ/g of BW) of the proventriculus, gizzard, pancreas, liver, and small intestine is observed at 4.1, 3.9, 8.1, 4.6, and 7.9 days of age, respectively (Table 2), data that compare well with information from Sell (1996). Batal and Parsons (2002a) found that the ME(n) and the apparent digestibility of starch, fat, and selected amino acids of a corn-soybean meal diet was low at 2 days and reached a plateau at 14 days of age (Table 3). Lilburn (1998) indicated that overall digestibility of lipids in chicks for the first 3 to 5 days of age varies from 69% to 80% with the higher values corresponding to unsaturated fats. Therefore, proper lipid sources can be used successfully in prestarter diets for poultry.

Starch digestibility is critical for understanding energy utilization because the starch content of a
Table 2. Changes with age in relative weights of digestive organs of the chick (% BW).

<table>
<thead>
<tr>
<th>Age, days</th>
<th>Day of maximal relative growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Proventriculus</td>
<td>0.87</td>
</tr>
<tr>
<td>Gizzard</td>
<td>5.28</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.15</td>
</tr>
<tr>
<td>Liver</td>
<td>2.55</td>
</tr>
<tr>
<td>Small intestine</td>
<td>2.74</td>
</tr>
</tbody>
</table>

1Gracia et al. (2003a).

P<0.001 with age (0, 4, 8, 15, 21 days).

Table 3. Influence of age on apparent fecal digestibility of nutrients in New Hampshire x Columbian male chicks.1,2

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>ME(n)(kcal/kg DM)</th>
<th>Apparent fecal digestibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>2.970c</td>
<td>Starch: 93c, Fat: 61b, Lys: 78d, Met: 80c</td>
</tr>
<tr>
<td>3-4</td>
<td>3.085c</td>
<td>Starch: 93c, Fat: 58b, Lys: 81c, Met: 82c</td>
</tr>
<tr>
<td>7</td>
<td>3.185b</td>
<td>Starch: 97c, Fat: 59b, Lys: 85b, Met: 87b</td>
</tr>
<tr>
<td>14</td>
<td>3.429b</td>
<td>Starch: 99a, Fat: 74b, Lys: 89b, Met: 92a</td>
</tr>
<tr>
<td>21</td>
<td>3.426c</td>
<td>Starch: 99b, Fat: 73b, Lys: 89b, Met: 92b</td>
</tr>
<tr>
<td>SEM</td>
<td>26</td>
<td>Starch: 0.4, Fat: 1.3, Lys: 0.7, Met: 0.9</td>
</tr>
</tbody>
</table>

1Batal and Parsons (2002a).
2Diets with 5.5% added soy oil.

α-Methyl-amylose is produced in excess of requirements (Moran, 1985; 1992), but several reports indicate that starch digestion at the end of the ileum of young birds is incomplete. Rogel et al. (1987) observed that in meal diets, fecal digestibility of wheat starch was 77.2% at 3 weeks and 97.8% at 6 weeks of age. Weurding et al. (2001) found that total tract digestibility of starch varied from 98.9% for tapioca pellets to 31.7% for raw potato starch, with intermediate values for cereals (93.8 to 98.3%) and legume grains (74.5 to 81.5%). Mateos et al. (2002) and Gracia et al. (2003a) observed that total tract digestibility of starch and ether extract in broilers increased with age in both corn- and barley-soybean meal diets (Table 4). Similar results have been reported by Yuste et al. (1991), Batal and Parsons (2002a, b) and Gracia et al. (2003b). Enzyme accessibility to starch is determined by the viscosity of gut contents and the nature and structure of the starch granules. In general, starch in small granules is hydrolyzed more rapidly than starch in large granules. Also, starches with high amylose content are less susceptible to amylase attack than starches with low amylose content. Therefore, rice might be a candidate for prestarter diets for chicks because its starch is very accessible and mostly of type A (easily digested compact starch with no free space left for water), granule size is very small, the amylose content is lowest among cereals, and the grain has very low content of β-glucans and xylans. A recent study conducted in our laboratory (González-Alvarado et al., unpublished) has confirmed the potential of rice as an energy source in prestarter feeds for broilers.

In two different trials we studied the influence of the main cereal of the diet (60% corn vs 60% rice), processing of the cereal portion of the diet (raw vs cooked and rolled) and the inclusion of insoluble fiber sources (none vs 3% soy hulls vs 3% oat hulls) for broilers from 1 to 21 days of age. Rice feeding consistently improved feed conversion in both trials, but no differences between cereals were observed for feed intake or daily gain (Table 5). The data indicate that rice is well utilized by the chick and that its metabolizable energy content is approximately 3 to 5% higher than that of corn.

Table 4. Influence of age on starch and ether extract fecal digestibility in broilers (%).

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>ME(n =10)</th>
<th>Starch</th>
<th>Ether extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>95.0</td>
<td>95.0</td>
<td>96.7</td>
</tr>
<tr>
<td>8</td>
<td>95.8</td>
<td>96.2</td>
<td>97.2</td>
</tr>
<tr>
<td>15</td>
<td>96.2</td>
<td>96.7</td>
<td>97.0</td>
</tr>
<tr>
<td>21</td>
<td>97.2</td>
<td>97.2</td>
<td>74.7</td>
</tr>
</tbody>
</table>

1Mateos et al. (2002). Corn-soybean meal diet with 2.7% lard.
2Gracia et al. (2003a). Barley-soybean meal diet with 6% lard.

Table 5. Influence of type of cereal in the diet on feed conversion (g of feed/g of gain) of broilers from 1 to 21 d of age.1

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 4</td>
<td>1.34</td>
<td>1.32</td>
</tr>
<tr>
<td>4 to 8</td>
<td>1.23a</td>
<td>1.16a</td>
</tr>
<tr>
<td>8 to 14</td>
<td>1.33a</td>
<td>1.28a</td>
</tr>
<tr>
<td>14 to 21</td>
<td>1.45</td>
<td>1.41</td>
</tr>
</tbody>
</table>

1González-Alvarado et al. (unpublished data). Diets based on 60% of raw or cooked cereal.
2Means within a column (for each trial) with no common superscript differ significantly (P<0.05).
Heat processing of ingredients and diets

Heat is usually applied to pellet poultry feeds and also to inactivate thermolabile antinutritional factors contained in some raw materials such as soybeans. Recently, expanded feeds (110°C to 120°C for 5 sec) have been introduced into the market because of beneficial effects on feed hygiene, nutrient digestibility, and broiler productivity (Fancher et al., 1996; Mateos and Lázaro, 2001). The information available on the influence of heat processing on digestive physiology and poultry performance is scarce and contradictory (García et al., 1998; Mateos et al., 2002). Heat processing disrupts feed structure, facilitating the access to nutrients by digestive enzymes. However, heat processing also shifts the site of starch digestion, facilitates Maillard reactions, and solubilizes part of the starch and of the NSP, increasing digesta viscosity. Plavnik and Sklan (1995) have found that dry extrusion or expansion of a corn diet improved the ME(n) by 1.5 to 3%, primarily due to an improvement in fatty acid digestibility but no effect was found by Vukic Vranjes et al. (1994). Commercial information indicates that heat processing of barley and wheat diets increases the incidence of wet litter and that the judicious use of appropriate enzymes reduces the condition (Nissinen et al., 1993), data which are corroborated by our own results (Lázaro et al., 2003a, b, 2004). García et al. (1998) studied the influence of heat processing of barley (cooked at 99°C for 50 min) and exogenous enzymes (β-glucanase and xylanase) on broiler performance at 42 days (Table 6). Heat processing of barley improved daily gains at 7 days but the effects disappeared thereafter. Enzymes improved performance at all ages but no interaction of processing and enzymes was detected for any trait. Similar results have been obtained by Mateos et al. (2002), working with raw and cooked corn (Amandus Kahl, Reinbeck, Germany) diets (Table 7). In a recent trial, González-Alvarado et al. (unpublished) have studied the influence of including heat processed corn or rice in the diet on performance of broiler chicks. The diets contained 60% cereal either raw or cooked (90°C for 50 min and then rolled). Heat processing had little effect on productivity at any age but improved feed conversion in diets based on rice, although not in diets based on corn (Table 8).

### Particle size, feed form, and whole grains

The benefits of feed processing have long been recognized by the feed compound industry. Physical

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**Table 6. Influence of barley processing and enzyme supplementation on performance of broilers.**

<table>
<thead>
<tr>
<th></th>
<th>0-7 days</th>
<th></th>
<th>0-42 days</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADG (g)</td>
<td>FC (g/g)</td>
<td>ADG (g)</td>
<td>FC (g/g)</td>
</tr>
<tr>
<td>Enzymes²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14.6</td>
<td>1.20</td>
<td>53.7</td>
<td>1.71</td>
</tr>
<tr>
<td>500 ppm</td>
<td>15.5</td>
<td>1.15</td>
<td>57.4</td>
<td>1.65</td>
</tr>
<tr>
<td>Heat processing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>14.3</td>
<td>1.18</td>
<td>55.5</td>
<td>1.69</td>
</tr>
<tr>
<td>Heated</td>
<td>15.5</td>
<td>1.18</td>
<td>55.6</td>
<td>1.68</td>
</tr>
<tr>
<td>SEM (n = 12)</td>
<td>0.657</td>
<td>0.037</td>
<td>1.19</td>
<td>0.027</td>
</tr>
<tr>
<td>Enzymes³</td>
<td>0.05</td>
<td>0.01</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Heat processing</td>
<td>0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1García et al. (1998).
2Xylanase and β-glucanase complex from *Aspergillus niger*.
3Average value for micronized and expanded barley.

**Table 7. Influence of heat processing, enzyme supplementation, and age on fecal starch digestibility of corn diets for broilers (%).**

<table>
<thead>
<tr>
<th>Broiler age (days)</th>
<th>4</th>
<th>8</th>
<th>15</th>
<th>21</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat processing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>94.2b</td>
<td>94.9b</td>
<td>95.7b</td>
<td>97.1</td>
<td>95.5</td>
</tr>
<tr>
<td>Cooked²</td>
<td>95.8a</td>
<td>96.7a</td>
<td>96.7a</td>
<td>97.3</td>
<td>96.5</td>
</tr>
<tr>
<td>Enzymes³</td>
<td>0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>500 ppm</td>
<td>94.8</td>
<td>95.9</td>
<td>96.2</td>
<td>97.3</td>
<td>96.1</td>
</tr>
<tr>
<td>99°C for 50 min followed by rolling.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protease, xylanase, and α-amylase complex.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>²Means in a column differ significantly (P&lt;0.05).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age effect (P&lt;0.01).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 8. Influence of heat processing of the cereal portion of the diet on broiler performance from 0 to 21 d of age.**

<table>
<thead>
<tr>
<th>Cereal Process²</th>
<th>Age of broilers (days)</th>
<th>0 to 4</th>
<th>0 to 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADG⁴</td>
<td>FC⁵</td>
<td>ADG</td>
</tr>
<tr>
<td>Corn Raw</td>
<td>14.5⁶</td>
<td>1.31ab</td>
<td>31.2</td>
</tr>
<tr>
<td>Corn HP²</td>
<td>13.5⁶</td>
<td>1.36⁶</td>
<td>32.0</td>
</tr>
<tr>
<td>Rice Raw</td>
<td>13.9⁶</td>
<td>1.34⁶</td>
<td>32.4</td>
</tr>
<tr>
<td>Rice HP</td>
<td>13.9⁶</td>
<td>1.30⁶</td>
<td>31.9</td>
</tr>
<tr>
<td>SEM (n = 18)</td>
<td>0.125</td>
<td>0.019</td>
<td>0.53</td>
</tr>
<tr>
<td>Main effects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cereal</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Heating</td>
<td>0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1González-Alvarado et al. (unpublished data).
2Cooked (90°C; 50 min) and rolled.
3Average daily gain, g.
4Feed intake per g of body weight gain.
5Significant interaction: cereal x HP for ADG (P<0.001) and FC (P<0.05) from 0 to 4 days and for FC (P<0.05) from 0 to 21 days.
structure of the feed (wet feeding, particle size, feed form, and inclusion of whole grains) influences GIT structure, composition of the microflora, nutrient digestibility, and feed intake. Fine grinding of feedstuffs is a common practice because small particle size improves nutrient digestibility and facilitates the process of pelleting (Douglas et al., 1990; Lott et al., 1992; Kilburn and Edwards, 2001). However, finely ground cereals might be detrimental for mucosal cell growth and motility of the GIT. Fine particles produce atrophy of the gizzard, a major regulator of intestinal motility (Nir et al., 1994; Jones and Taylor, 2001). Nir et al. (1994) theorized that large particles enhance GIT motility and stimulate peristalsis and digesta backflow whereas finely ground particles reduce the reflux of the digestive contents, resulting in more nutrients passing undigested to the hindgut. In a recent study, Kilburn and Edwards (2004) have found that coarse soybean meal increases bone ash of broiler chicks, probably through an improvement in mineral utilization, and also improves growth and feed efficiency when used in semipurified diets.

In commercial practice most diets for broilers and turkeys undergo some type of processing. It is widely accepted that pelleting enhances feed value by making more nutrients available for growth. Two areas of interest in this respect are the influence of pellet quality on feed intake by young poults and chicks and on performance of growing and finishing birds. Quality and characteristics of the pellets define the ingestion of prestarter feeds by chicks and poults (Picard et al., 2000). In fact, the industry has started to manufacture micropellets or small crumbles of uniform size to maximize intake and improve performance at early ages (Martins, 2003). It is widely accepted that pellet quality of corn-based diets for broilers should provide more than 60% to 65% intact pellets at feeder level to maximize bird performance. However, there is a clear trend toward increasing the energy concentration of diets for broilers and turkeys to maximize growth, which often results in the inclusion of higher levels of supplemental fat. Usually, an increase in the dietary fat level results in a reduction in pellet quality. Consequently, efforts to increase the energy intake of birds through fat supplementation may be partially offset by a reduction in pellet quality. Therefore, any addition of fat to the diet of broilers to improve performance must not compromise pellet quality. On the other hand, a problem frequently found in commercial operations when wheat is the main cereal of the diet is the excessive hardness of the pellets, which reduces feed intake and increase wastage by the bird.

Feeding whole grains to poultry has been a common practice in Europe for the last 50 years. In fact, some poultry integrators are diluting broiler rations with up to 25% whole wheat to reduce feed cost of poultry diets. Plavnik et al. (2002) observed that the inclusion of 20% whole wheat in diets for broilers improved body weights (2,494 vs 2,431 g; P<0.05) and feed conversion (1.82 vs 1.93 g/g; P<0.05) at 7 weeks and increased gizzard weight (16.5 vs 15.0 g/kg BW; P<0.05). Sвиhus et al. (1997) observed that duodenal digesta from chickens fed whole grain had similar particle size to digesta from chickens fed ground grain. In fact, Sвиhus et al. (2002) observed that replacement of ground wheat with whole wheat increased ileal starch digestibility (93% to 99%) indicating that whole grain feeding may improve bird performance by stimulating gizzard development and enhancing enzyme production.

Crude fiber, nonstarch polysaccharides, and enzymes

An excess of fiber in feeds might impair nutrient digestibility and feed efficiency. Dietary fiber often increases endogenous losses leading to a decrease in ileal digestibility of starch, protein, and lipids (Souffrant, 2001). As a consequence, current practical diets for prestarter feeds are based on low fiber ingredients such as corn, wheat, and high protein soybean meal. However, dietary fiber is a heterogenous class of components differing in structure and physiological properties. In general terms, soluble fiber increases intestinal transit time, delays gastric emptying, increases pancreatic secretion, and slows absorption, whereas insoluble fiber decreases transit time and enhances water-holding capacity (Montagne et al., 2003). Recent data indicate that adequate type and quantity of fiber could reduce digestive disturbances and improve the adaptation of the GIT of monogastric animals to current production systems (Graham and Aman, 1991; Hetland and Sвиhus, 2001; Mateos et al., 2001b; Hetland et al., 2003; Sklan et al., 2003). For example, proventricular hypertrophy and poor gizzard development has been linked to the use of low fiber diets (Riddell, 1976). Montagne et al. (2003) indicate that, depending on nature and physiological factors, dietary fiber may improve gut health, or alternatively enhance gut perturbation and subsequent diarrhoea.
in young animals. Therefore, more studies are needed to find the maximum and minimum levels of dietary fiber to be included in diets for poultry, especially at young ages. In fact, the British Society of Animal Science has recently proposed a minimum of crude fiber and of neutral detergent fiber in diets for young pigs (BSAS, 2003) but no studies have been conducted with young birds. We have studied the influence of including different sources of insoluble dietary fiber in diets for broiler chicks (González-Alvarado et al., unpublished). Two control diets consisting of 60% corn or rice (either raw or cooked) and soy protein concentrate were formulated. The experimental diets included 3% of either soy hulls or oat hulls at expense of an inert material. The inclusion of the fiber sources consistently improved broiler performance at 21 days of age (Table 9). In a second test, diets low in fiber (1.5% crude fiber and 3.6% NDF) based exclusively on rice and soybean protein concentrate were formulated. The test diets consisted of adding 3% oat hulls (a source of lignified insoluble fiber) or soy hulls (a source of non-lignified insoluble fiber) at expense of an inert material. The inclusion of the two sources of fiber to the rice-soy protein concentrate diet improved feed conversion at early stages of growth and improved average daily gain at 21 days of age (Table 10). The inclusion of either soy hulls or oat hulls to the low fiber diets increased relative gizzard weight (% BW) (1.786c vs 2.407a and 1.868b for control, oat hulls and soy hulls-including diets; P<0.001) and relative total digestive weight (% BW) (10.23b vs 10.76a vs 10.76ab%; P<0.01). Also, soy hulls but not oat hulls increased intestinal viscosity at 21 days of age (3.38 vs 3.36 vs 3.76 cP for control, oat hulls, and soy hulls diets, respectively; P<0.05).

Table 9. Influence of fiber inclusion in the diet on performance of broilers.1

<table>
<thead>
<tr>
<th>Fiber source</th>
<th>ADG (g)</th>
<th>FI (g/day)</th>
<th>FC (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>13.8</td>
<td>18.7</td>
<td>1.36</td>
</tr>
<tr>
<td>Oat hulls, 3%</td>
<td>14.1</td>
<td>18.4</td>
<td>1.31</td>
</tr>
<tr>
<td>Soy hulls, 3%</td>
<td>14.0</td>
<td>18.5</td>
<td>1.32</td>
</tr>
<tr>
<td>SEM (n = 24)</td>
<td>0.109</td>
<td>0.23</td>
<td>0.016</td>
</tr>
<tr>
<td>P1</td>
<td>NS</td>
<td>NS</td>
<td>0.05</td>
</tr>
<tr>
<td>0-21 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>30.77</td>
<td>42.7</td>
<td>1.39</td>
</tr>
<tr>
<td>Oat hulls, 3%</td>
<td>32.2a</td>
<td>43.1</td>
<td>1.34</td>
</tr>
<tr>
<td>Soy hulls, 3%</td>
<td>32.7a</td>
<td>44.2</td>
<td>1.35</td>
</tr>
<tr>
<td>SEM (n = 24)</td>
<td>0.046</td>
<td>0.66</td>
<td>0.010</td>
</tr>
<tr>
<td>P1</td>
<td>0.01</td>
<td>NS</td>
<td>0.001</td>
</tr>
</tbody>
</table>

1Gonzalez-Alvarado et al. (unpublished data).
2Significance of contrast: none vs hull inclusion.

Table 10. Effects of adding fiber sources to a low fiber diet on broiler performance at 21 days.3

<table>
<thead>
<tr>
<th>Fiber source</th>
<th>ADG (g)</th>
<th>FI (g/day)</th>
<th>FC (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>30.7</td>
<td>42.9</td>
<td>1.39</td>
</tr>
<tr>
<td>Oat hulls, 3%</td>
<td>33.1</td>
<td>43.5</td>
<td>1.31</td>
</tr>
<tr>
<td>Soy hulls, 3%</td>
<td>33.4</td>
<td>44.8</td>
<td>1.34</td>
</tr>
<tr>
<td>SEM (n = 6)</td>
<td>0.92</td>
<td>1.32</td>
<td>0.019</td>
</tr>
<tr>
<td>P1</td>
<td>0.05</td>
<td>NS</td>
<td>0.001</td>
</tr>
</tbody>
</table>

3Gonzalez-Alvarado et al. (unpublished data).
3Significance of contrast: none vs hull inclusion.

Enzymes reduce feed cost when viscous cereals are the major source of energy. Non-starch polysaccharides, specifically the soluble fraction, have a negative impact on digestion and absorption of nutrients in poultry. The mechanisms by which NSP reduce broiler performance are not well understood. Soluble NSP increase digesta viscosity and reduce the accessibility of enzymes to starch, protein, and lipids of the diet. Added enzymes improve bird performance by increasing nutrient digestibility and feed intake, and balancing intestinal fermentation. Lázaro et al. (2003a, b) have indicated that for laying hens the beneficial effects of supplementary enzymes are mostly due to improved nutrient digestibility, whereas in broilers an increase in feed intake is also important. Fat is usually the nutrient whose digestibility is most benefited by added enzymes, although improvements of up to 8% for starch and of 19% for nitrogen digestibility have been reported in barley diets (Hesselman and Åman, 1986).

The available information indicates that enzymes improve nutrient digestibility and poultry performance and that the beneficial effects are more pronounced in broilers fed viscous grain diets than in broilers fed corn-based diets. Also, enzymes are more effective when heat processed ingredients or diets such as expanded feeds are used. Information on oligosaccharidases is scarce; and research effort to increase its use in diets based on soybeans protein meals and legumes is warranted.

Nutrition, immunity and enteric disorders

Birds at hatch have an immature immune system and are susceptible to enteric disorders associated with exposure to pathogens (Bar-Shira et al., 2003). Changes in GIT conditions due to disease have a significant impact on the efficiency and requirements
for nutrients in the chick, because bacterial challenges redirect nutrients from growth toward host defense (Obled, 2002). Potential limiting amino acids for immune protein synthesis are unknown, but lysine is probably not limiting (Klasing and Leshchinsky, 2000). Rowlands and Gardiner (1998) indicate that in humans, certain amino acids (glutamine, arginine, and ornithine), fatty acids (short chain and n-3 fatty acids), and nucleotides (DNA) might enhance intestinal integrity and support immune function. There is evidence in humans and pigs that luminal glutamine benefits mucosal permeability, is used for gluconeogenesis, and is a major fuel and building block for synthesis of nucleotides in rapidly proliferating cells, such as those of the immune system and intestinal mucosa (Wu, 1998; Obled, 2002). Whether an exogenous supply of these nutrients will enhance the mucosal barrier and support immune function in the bird is controversial at the present time.

Necrotic enteritis is an acute, infectious, non-contagious disease caused by the overgrowth of Clostridium perfringens that affects the lining of the digestive tract in chicks from 2 weeks to 6 months of age. Factors that precipitate outbreaks of the disease include management stress, subclinical coccidiosis and abrupt changes in dietary formulation. Historically, in-feed antibiotics have been used for the treatment and prevention of NE and there is strong evidence that banning antibiotics has contributed to an increase in the incidence of the disease.

Diet has a marked effect on the development of the microflora in the alimentary tract of the chick. Two major dietary factors that predispose flocks to NE are the use of cereal grains that increase the viscosity of digesta and the high levels of crude protein in the diet (Drew et al., 2004). Kaldhusdal and Skjerve (1996) observed that the inclusion of corn in wheat or barley diets for broilers contributed to the prevention of the disease. In addition, dietary lactose reduced intestinal counts of clostridia while sucrose, glucose, and fructose were associated with an increase (Riddell and Kong, 1992). Bedford (2000) hypothesized that the proliferation of C. perfringens is facilitated by the presence of large quantities of dietary protein in the ceca. If this is the case, viscous diets will increase the quantities of nitrogen that escape digestion resulting in a more frequent occurrence of NE (Al Sheikhly and Al Saieg, 1980; Riddell and Kong, 1992). When the incidence of enteric disorders is high, an increase in the use of high quality fish meal and of synthetic amino acids, and a reduction of the protein content of the diet is recommended (Mateos et al., 2001).

In general, broilers fed wheat or other high NSP grains are more susceptible to NE than broilers fed corn (Ridell and Kong, 1992; Kaldhusdal and Skjerve, 1996). However, the addition of pentosanases to wheat diets did not affect mortality due to NE, which indicates that other factors are responsible for the increase in clostridia counts observed in the GIT of birds fed wheat. Further studies are needed to investigate the influence of different dietary fiber sources on GIT motility, intestinal digesta movements, and the occurrence of enteric diseases.

It has been proposed that the control of antinutritional factors present in the diet, including mycotoxins, lectins and trypsin inhibitors might help to reduce enteric disorders. The use of soybean meal and soy products has increased in Europe because of restrictions imposed on the utilization of animal proteins and more than 40% of soy products are frequently used in turkey diets. Yet, soybeans are processed the same way today as they were 60 years ago and no methods have been implemented to remove the oligosaccharides present in the meal. In addition, full-fat soybeans are being included at high levels in all-vegetable diets, with inadequate control of antinutritional factors in many cases. Many nutritionists accept concentrations of 6 to 8 mg/kg of trypsin inhibitor in treated soybeans. However, Clarke and Wiseman (2001) have indicated that this level should be reduced to less than 4 mg/kg in young animals. Van der Klis and Jansman (2002) have estimated that the presence of 5.7 mg of trypsin inhibitor per kg of diet in piglets increases the percentage of energy required for maintenance from 5.5 to 13.1% (Table 11).

| TIA, mg/kg | Endogenous N excretion, g/d | Endogenous N synthesis, g/d | Energy expended, KJ/d | Energy required, %
<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.7</td>
<td>38.6</td>
<td>174</td>
<td>5.5</td>
</tr>
<tr>
<td>1.9</td>
<td>16.3</td>
<td>65.2</td>
<td>293</td>
<td>9.3</td>
</tr>
<tr>
<td>5.7</td>
<td>23.0</td>
<td>91.9</td>
<td>414</td>
<td>13.1</td>
</tr>
</tbody>
</table>

1Van der Klis and Jansman (2002).
2Trypsin inhibitor activity.
3Excreted ileal endogenous N (g/d).
4Total synthesised endogenous N (g/d).
5Energy costs of endogenous protein synthesis (KJ/d).
6Percentage of maintenance requirements.
Conclusions

Enteric diseases are complex and affected by many factors, such as subclinical coccidiosis, stresses, lack of hygiene, and immunodepression, but dietary changes that improve gut health and stimulate gizzard development and motility might help to overcome digestive upsets. Coarse grinding, mash feeds, low wheat and protein diets, enzyme supplementation, inclusion of whole grains and a minimum amount of a convenient fiber source are some of the solutions proposed in this respect. In addition, the inclusion of essential fatty acids, emulsifiers, organic acids probiotics, and prebiotics, as well as immune enhancers, is also recommended. Most of these techniques are not sufficiently refined at present and further research is needed. Adequate management in terms of preventive vaccination programs, reduction of stresses, and improvement of hygienic conditions, together with dietary changes minimize the incidence of enteric disorders in antibiotic-free fed flocks.

Removal of in-feed antibiotics from feeds is likely to increase production costs moderately, mostly because of the increase of enteric disorders including NE, but efficient production is still feasible without antibiotic use.

References


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Svihus, B., E. Juvik, and A. Krogdahl. 2002. The increase in starch digestibility when ground wheat is replaced with whole wheat in broiler diets is associated with an increase in jejunal bile acid concentration and amylase activity. Poult. Sci. 80 (Supl. 1):57-58 (Abstr.).
Reproductive responses to Sel-Plex® organic selenium in male and female broiler breeders: impact on production traits and hatchability

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Growth selection and reproduction in broiler breeders

We expect a lot from modern broiler breeders. These highly growth-selected birds must pass the genetics for fast and efficient growth to as many offspring as possible. While crossbreeding and selection programs have resulted in annual improvements in broiler growth, breast muscle yield, feed efficiency and disease resistance, there is a negative relationship between body weight and egg production in broiler breeders (Robinson et al., 1993). While specialized genetic selection has meant that egg production is not remarkably different from what it was a few years ago, hatching egg producers have had to work hard at fine-tuning strain specific procedures for nutrient allocation and photoperiod management.

Achieving success with broiler breeder management is like hitting a moving target. Modern broiler stocks have been reported to grow at 4.6 times the rate of a 1957 random-bred strain due to increased genetic potential (Havenstein et al., 2003a). The 6-fold improvement in carcass yield of 2001 stocks fed a 2001 diet compared to 1957 stocks fed a 1957 diet is 85-90% due to genetics, and 10-15% due to nutritional changes (Havenstein et al., 2003b). While broiler 42-day body weight is increasing each year, the 42-day target body weight for male and female broiler breeders has remained the same, or even decreased (Rustad and Robinson, 2002). In 1979, Hubbard male and female breeders were approximately 50% of the 42-day broiler weight. In 2001, this percentage had decreased to 36.1 for males and 30.3 for females. In essence, the degree of feed restriction has continued to increase while there is increased competition for a reduced feed allocation. Complications in managing this increased growth efficiency is further complicated by the development of ‘yield’ varieties, carrying increased amounts of breast muscle, often on a smaller carcass frame. As selection for broiler breeder egg production is not as heritable or profitable as selection for growth traits, there are continued increases in the growth potential while egg production is not emphasized. As a result, Whitehead (2000) indicates that geneticists continually compound the problem by breeding a bird that, if allowed to exist in its freely expressed adult state, is completely unfit for life.

Understanding the ovarian function of the chicken and its interaction with nutritional status, age, and strain is likely the most important issue affecting poultry breeding companies today. The process involves the conversion of genetic, environmental, and nutritional cues into a cascade of signals from the neuroendocrine system. These signals must be integrated and responded to by the organs and tissues primarily involved in reproduction, which will in turn produce more signals for both local and distant activities. The resulting eggs produced are the net result of the bird’s attempt to coordinate the demands its body and environment have placed on it.

The ability of an embryo to survive the incubation process relies on a balance between hatchery management and breeder management. Specific feed ingredients, bird age, and flock management decisions can directly affect semen quality, the oviduct environment, and the egg environment. These factors combine to influence the potential of the egg to be fertile and ultimately to hatch.
Reproduction in the broiler breeder

The reproductive system of the laying hen is comprised of many organs. The list includes the hypothalamus, the anterior pituitary, the ovary, the oviduct, the liver and the skeletal system. Small follicle steroidogenesis, particularly estrogen production, is responsible for transforming a pullet into a hen. As plasma levels of estrogens increase, externally visible features include reddening and enlargement of the comb and wattles, a prenuptial feather molt (feather drop) and a widening of the pubic bones to permit egg passage. Internally, estrogen stimulates liver production of egg yolk lipids with a significant change in the color and size of the liver. Finally, the oviduct enlarges and becomes a secretory organ for deposition of albumen.

The male focuses on quantity rather than quality when it comes to sperm production. The hen then must screen out unsuitable sperm in order to guarantee production of high quality chicks. Mature sperm spend the majority of their time in the oviduct. Following mating, the hen will store sperm in the highly specialized microenvironments of the sperm storage tubules (SST) are located in the vaginal region of the oviduct. Only about 1 to 2% of the originally inseminated sperm enter the SST (Bakst et al., 1994), where they exist in a near-dormant state. The survival of the sperm to the point of insemination depends on a combination of sperm quality and the ability of the hen to provide a safe environment for the sperm.

The hormone messages being relayed between the ovary and the hypothalamic and pituitary control centers are altered by feed intake. Besides affecting follicle formation, and reproductive control, feeding level can alter the viability of the embryo through changes to the egg and to the early maturation process. Excess nutrients are diverted into liver lipids, excess ovarian follicle development, and as abdominal fatpad (Etches, 1996). It can be a vicious cycle, with obesity continuing to worsen as the rate of egg production remains low and/or goes into early decline due to excess feed intake. The ovaries of growth-selected strains are particularly sensitive to overfeeding during the sexual maturation process (Renema et al., 2003).

Body weight in broiler breeder hens has been reported to be negatively correlated with duration of fertility and fertile egg production (Bilgili and Renden, 1985). Ultimately, reduced chick production in overfed broiler breeders is the culmination of poor egg production combined with reduced fertility, hatch of fertile, and embryonic viability (Yu et al., 1992).

The female oviduct environment can be hostile to sperm despite their existence in the SST within the oviduct wall. Duration of fertility (measured by monitoring fertility in consecutive eggs) can be reduced under conditions of overfeeding (Katanbaf et al., 1989b; Goerzen et al., 1996). It is known that fewer sperm survive in some bird strains and when excess feed is used (Renema et al., 2001), but it is not clear how the surviving sperm are affected, and if the remaining sperm are of similar quality to the ones originally placed.

Factors affecting hatching egg quality

There are many factors that can affect the potential of the embryo to survive incubation and generate a quality chick. Some of these factors are out of our control, such as hen age, and others can be manipulated through management decisions, such as egg size and hatchery environment. It can be difficult to formulate diets to optimize egg production, fertility, and hatchability as little is known about the nutritional requirements of the embryo (Leeson and Summers, 1991). Dietary vitamin levels are increased in the diet with the hope they will also be increased in the egg. Yet there can be adverse reactions with this type of approach, as some vitamins have antagonistic relationships with others. Furthermore, there can be stability issues for long-term storage, as well as for feed processing procedures. With current genetic stocks, if the chick hatches in a weakened state due to a vitamin or mineral deficiency, it is more likely to succumb to disease now than with previous stocks. Growth-selected stocks have low immunoresponsiveness (Siegel et al., 1984) due to either inadvertent or intentional negative selection pressure combined with growth efficiency selection. The developing embryo is especially sensitive to vitamin deficiency, which will result in death, malformation or some other atypical response (Leeson and Summers, 2001).

During embryo development, oxidative metabolism increases substantially over the incubation period and especially in the last few days before hatch (Freeman and Vince, 1974). This normal respiration related to embryo growth results in the production of free radicals, which can cause tissue damage through lipid peroxidation, with polyunsaturated fatty acids being especially vulnerable (Surai, 1999). The chick has
developed effective antioxidant pathways to prevent damage. The primary defense mechanism is a group of three enzymes (superoxide dismutase, glutathione peroxidase, and catalase), which convert free radicals produced by cellular respiration into less harmful alcohols (Ursiny et al., 1997). A second level of defense are the natural antioxidants – vitamin E, carotenoids, ascorbic acid, and glutathione, which protect the developing chick (Surai, 1999). During the last week of incubation, fat-soluble antioxidants are moved into the liver and yolk sac membrane. The major fat soluble antioxidant, vitamin E, moves from the yolk to the embryo tissue at this time (Gaal et al., 1995). Ascorbic acid (vitamin C) is the major water-soluble antioxidant, and is produced in the yolk sac membrane before transport to tissues like the brain (Surai, 1999). This helps protect membrane lipids during the large metabolic effort of hatching. The third level of antioxidant defense is the generation of enzymes that rebuild damaged membranes (Surai and Sparks, 2001).

ROLE OF DIETARY SELENIUM

Selenium is normally provided in the diet in the form of inorganic sodium selenite. An organic form can be provided (Sel-Plex®), which is selenium yeast. Yeast, like plants, form selenoamino acids and other organic selenocompounds that exist in very reduced form in comparison to the highly oxidized inorganic selenium forms (selenite and selenate). Organic minerals are transported intact and retained better in target tissues or organs. Higher selenium in eggs reflects increased antioxidant properties of the egg during storage, therefore preserving the egg for incubation and potentially increasing hatchability. Cantor (1997) and Paton et al. (2000b) found that eggs from Sel-Plex®-fed chickens were significantly higher in selenium than eggs from sodium selenite-fed chickens. Organic selenium has a vitamin E-sparing action through its involvement in vitamin E retention in the plasma and through involvement with the primary enzymatic defense system of the embryo against lipid peroxidation. In fact, supplementing organic selenium to breeder diets has been shown to increase levels of other antioxidants (vitamin A, E and carotenoids) in the egg (Surai and Sparks, 2001).

The protective effects of organic selenium are especially apparent during the highly oxidative state of late incubation and the first few days after hatch. Selenium is an integral part of the antioxidant enzyme glutathione peroxidase (GSH-Px) as well as a component of many other selenoenzymes. Oxygen metabolism produces free radicals, which have potentially toxic effects on all biological molecules (Surai, 2000). Glutathione peroxidase aids in the removal of oxidative compounds in the form of hydrogen peroxide and hydroperoxides from the cell (Burk, 1989). Buildup of these substances can impair cell membrane structure and function, and once the membrane is damaged decreased productivity and reproductive performance can result (Surai, 2000). Selenoamino acids have been shown to have higher bioavailability than traditional inorganic sources commonly used for dietary supplementation. They are actively absorbed in the intestine compared to the passive absorption of inorganic selenium (Surai, 2002). Furthermore, selenomethionine and selenocysteine can be incorporated non-specifically into structural proteins (particularly muscle tissue) during protein synthesis. Selenomethionine can be substituted for methionine during protein synthesis due to its similar structure (methionine contains a sulphur atom instead of a selenium atom). This critical difference between selenium sources allows the organic selenium compounds in Sel-Plex® to contribute to a selenium reserve to be available for prevention of lipid peroxidation (through GSH-Px) during stress conditions (Surai, 2002). In the broiler breeder, it also enables enhanced transfer of selenium from the hen to the embryo (Edens, 2002). Increased antioxidant uptake in the hen due to the maternal diet is linked to increased antioxidant concentrations in the developing chick (Surai et al., 1999).

The need for defense against oxidative damage is clear in the male, where antioxidant enzymes play a key role in maintaining the sperm cells (Surai et al., 1998). Sperm cells contain large amounts of poly-unsaturated fatty acids, which allow them to maintain flexibility relating to motility (Surai, 2002). However, this means they are also a target for lipid peroxidation. Cellular integrity is maintained by GSH-Px, other selenoenzymes and vitamin E, which protect the cell membranes from oxidative damage (Flohe and Zimmermann, 1970).

Some recent research has demonstrated that the inclusion of selenium in poultry diets enhances sperm numbers, and using an organic source (Sel-Plex®) reduces production of defective sperm, thereby having a positive effect on the fertilizing potential of the male (Edens, 2002). Little information is available regarding the effect of dietary selenium source on the reproductive efficiency of laying hens.
How does dietary selenium source affect the hen’s contribution to fertility? (Study 1)

Egg production and fertility decline with age. The decrease in hatchability and fertility associated with an increase in age might be due to the older hen’s inability to hold sperm in the SST (Fasenko et al., 1992). Furthermore, the sperm do not retain their viability as long in the SST of older hens, and are released in larger numbers from the SST (Bramwell et al., 1995). Quantification of fertility is determined by the occurrence rate of perivitelline holes caused by the sperm. In past research, killing the hen to obtain the newly ovulated ovum was the only way to determine sperm hole quantities. Bramwell et al. (1995) adapted the technique to use eggs for the determination of perivitelline sperm hole numbers.

This study examined the effects of selenium supplementation form and level on female reproductive performance and egg traits. Its intent was to determine the effects of organic (Sel-Plex®) and inorganic selenium supplementation in the laying hen diet on fertilization potential and egg traits.

METHODS

We housed 75 hens in individual laying cages at 61 weeks of age. Three dietary treatments were imposed, varying in selenium source and level. Twenty-five hens were fed a control diet, 25 were fed a diet enriched with inorganic selenium in the form of sodium selenite and 25 were fed a diet enriched with organic selenium in the form of the Alltech product, Sel-Plex®. All diets contain 19% CP and 2875 kcal ME/kg. The control diet had a selenium inclusion rate of 0.1 mg Se/kg, whereas both enriched diets contained a total of 0.3 mg Se/kg with an added 0.2 ppm Se coming from the organic or inorganic selenium source. The diets were fed for a 3-week period prior to insemination to ensure tissue saturation of the new dietary selenium forms and concentrations.

Following the 3-week acclimation period, all hens were artificially inseminated with 50 µL of neat, pooled semen collected from a group of 22 broiler breeder males (116 million sperm/dose). Eggs were collected from 2 to 7 days after insemination for the quantification of perivitelline layer sperm holes. Eggs traits (weight, specific gravity, yolk and dry shell weight) were measured at 30 and 60 days from the start of dietary treatments.

The sperm penetration assay of Bramwell et al. (1995) was used to quantify the perivitelline layer sperm holes. An approximately 1 cm² section of the perivitelline layer above the germinal disc was cut free, cleaned, mounted to a microscope slide, fixed, and stained with Schiff’s Acid reagent to generate a contrast with the sperm holes. The holes were counted at 100X magnification. The raw numbers and change in numbers over time were used as a representation of quantifiable fertilization potential.

OBSERVATIONS

Egg traits

The use of Sel-Plex® rather than sodium selenite as the dietary selenium source has previously been shown to increase shell breaking strength after 42 days in laying hens at 80 wk of age (Paton et al., 2000a). Our study found no significant differences in egg traits between the various treatments after 30 days on the diets. However, the Sel-Plex® treatment resulted in numerically the greatest positive change in shell quality during the 30 day period of this trial. Research with younger birds (26 wk of age) has indicated no difference in shell quality with the use of Sel-Plex® although the comparisons were made after only 28 days on the diet (Paton et al., 2000a). After 9 wks on the diets, shell weights were higher in the Sel-Plex® group than in the controls, while shell weights in the group given inorganic Se was intermediate (Table 1). Egg specific gravity, a measure of shell quality, was greater in the Sel-Plex® treatment than in either the control or selenite groups. While higher dietary selenium levels preserved shell quality to some degree, the organic selenium in Sel-Plex® proved to have a more substantial effect on the preservation of shell quality characteristics.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Shell weight (g)</th>
<th>Egg specific gravity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.43 a</td>
<td>1.0740 b</td>
</tr>
<tr>
<td>Sel-Plex®</td>
<td>5.68 b</td>
<td>1.0762 a</td>
</tr>
<tr>
<td>Inorganic Se</td>
<td>5.53 ab</td>
<td>1.0745 b</td>
</tr>
</tbody>
</table>

a,b Means within a column with no common superscript differ (P<0.05).

Perivitelline sperm hole assay

The Sel-Plex® treatment group had the highest mean number of sperm holes in the 2 to 4 days after insemination study period, while controls had the
lowest number (Table 2). Although neither the Sel-Plex® nor the control treatment were statistically different from the inorganic Se treatment, they were statistically different from each other. A similar relationship among the dietary treatments occurred for the 5 to 7 day period, and for a comparison over the entire 2 to 7 day period. The perivitelline sperm hole numbers declined at a similar rate among dietary treatment groups between the 2 to 4 day and the 5 to 7 day study periods. As the control group started with a lower number of sperm holes than either of the other two treatment groups, their final numbers at 7 days were very low. The Sel-Plex® and inorganic Se treatments both retained higher fertility potential throughout the sampling period allowing for a longer period of time between artificial inseminations. While not statistically different, the average number of sperm holes at the site of fertilization in the Sel-Plex® group was higher than that of the inorganic Se treatment. This indicates that the greater bioavailability of selenium in Sel-Plex® compared to inorganic Se may be advantageous for the fertility of the female based on changes to the oviduct environment. Selenium seems to play an important role in the maintenance of fertility in older laying hens. This is most likely due to the selenium-dependent GSH-Px improving the environment of the SST (Surai, 2000). The SST need to maintain a stable environment and the elimination or reduction of free radicals within the tubules is essential.

SUMMARY: STUDY 1

Selenium supplementation is beneficial to increasing and maintaining fertility and shell quality in older hens. Supplementation with the organic selenium in Sel-Plex® may have a greater impact on reproductive ability than inorganic sodium selenite. Factors such as age and length of exposure to the diet also play a role in the results of both this and past studies.

The form and quantity of dietary selenium appear to impact the oviduct environment of the hen. Fewer sperm are able to survive under low dietary selenium conditions (control) compared to conditions provided by supplementation with an organic selenium source (Sel-Plex®). Conditions in the SST may be central to the differences noted in the number of sperm being able to reach the site of fertilization. Through a combination of a more stable, antioxidant-free environment with a potentially slowed sperm metabolism, more sperm may be able to survive storage. Selenium source appears to influence the hen’s contribution to the fertility of the breeder flock.

Effects of dietary selenium source on the fertility and hatchability of broiler breeders (Study 2)

Supplying selenium to broiler breeders in the organic selenoamino acid form may have an important impact on poultry reproduction at the level of sperm formation, sperm storage, and in the hatching egg through increased protection from oxidative damage. This experiment was designed to provide information on the role of dietary selenium form on both female and male fertility. While there is evidence on a flock basis that selenium source affects broiler breeder female fertility, it is not as clear how these benefits are being expressed. Previous work suggests the use of an organic selenium source (Sel-Plex®) can lead to improved egg production, shell quality, sperm viability, and embryo survival. Egg shell quality may be enhanced through an altered efficiency of calcium metabolism, and sperm quality may be enhanced through protective antioxidant effects in the male and in the female oviduct. This study assessed some of these production and fertility traits in broiler breeders maintained individually.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Number of perivitelline sperm holes</th>
<th>Change in perivitelline sperm hole numbers between 2-4 &amp; 5-7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-4 days (P ≤ 0.004)</td>
<td>5-7 days (P ≤ 0.045)</td>
</tr>
<tr>
<td>Control</td>
<td>19.9^a</td>
<td>4.7^b</td>
</tr>
<tr>
<td>Sel-Plex®</td>
<td>55.8^a</td>
<td>10.3^c</td>
</tr>
<tr>
<td>Inorganic Se</td>
<td>40.7^a</td>
<td>9.5^b</td>
</tr>
</tbody>
</table>

^a,b Means within a column with no common superscript differ (P < 0.05).

^1Number of days after insemination.
The objective of this trial was to characterize specific effects of dietary selenium source on fertility and embryo viability aspects in commercial broiler breeder stocks. A female diet with no added selenium was used to identify the impact of dietary selenium. Inorganic and organic dietary selenium sources were compared to demonstrate the impact of differences in selenium accessibility and tissue storage on reproductive traits and embryo survival. Higher rates of production, fertility, and ultimately chick quality, would decrease the number of birds required to maintain current rates of production, as well as the overall cost of production.

METHODS

Ross 508 pullets were reared in a light tight facility following the breeder BW profile (Aviagen Inc). From photostimulation (22 wks of age) pullets were fed a selenium-free laying ration (No added Se), a standard ration containing sodium selenite (0.3 mg Se/kg), or a ration containing selenium yeast (0.3 mg Se/kg from Sel-Plex®). Thirty hens per treatment were inseminated weekly (from 30 wks) using pooled semen from males fed a standard, sodium selenite diet or a diet containing the same amount of Se from Sel-Plex®. Individual egg production to 58 wk, egg weight, egg specific gravity, and BW were recorded. At 35 and 57 wk of age, eggs from 2 to 5 days after insemination were subjected to the perivitelline sperm penetration assay to measure the number of sperm penetrations near the germinal disk. Eggs were incubated weekly and the hatch residue broken out to determine fertility, hatchability, and embryonic mortality.

OBSERVATIONS

Sperm management

Perivitelline sperm hole numbers of Sel-Plex® and selenite treatment eggs were similar. Both treatments had more sperm holes than eggs from unsupplemented hens by a factor of 2 to 3 (Table 3). Sel-Plex® supplementation improved maintenance of sperm numbers between the day 2 and the day 5 sampling. By day 5, Sel-Plex® eggs still had an average of 60 perivitelline sperm holes compared to 14 in control eggs, while selenite treatment eggs were intermediate (31 holes). These values represented a decline of 31% in apparent viable sperm population in Sel-Plex® birds between Day 2 and 5 after insemination compared to a 46% and 48% drop within non-supplemented and selenite-fed birds, respectively.

The ability to maintain a viable sperm population for as long as possible reduces necessary frequency of insemination. While selenium appears essential to allow the sperm into the oviduct, organic selenium in Sel-Plex® may have an advantage over inorganic selenium in keeping the sperm population stable and alive. This is especially important as the hens age and have a reduced sperm storage capacity at the uterovaginal junction (Goerzen et al., 1996).

Table 3. The effect of dietary selenium level and source on perivitelline sperm holes of eggs from broiler breeder hens.

<table>
<thead>
<tr>
<th></th>
<th>Number of perivitelline sperm holes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 days</td>
</tr>
<tr>
<td>No added Se</td>
<td>83b</td>
</tr>
<tr>
<td>Selenite2</td>
<td>150a</td>
</tr>
<tr>
<td>Sel-Plex®2</td>
<td>119ab</td>
</tr>
</tbody>
</table>

* Means within a column with no common superscript differ (P<0.05).

The males on the Sel-Plex® diet produced greater semen volume early in production, with an average of 0.36 ml/bird compared to 0.19 ml/bird in males on the selenite diet (36 weeks of age). At 56 weeks of age, this difference was no longer significant, but remained at nearly the same magnitude. The comparison was complicated at the later ages due to several small males dropping out of semen production part way through the trial (selenite treatment). Testes of all birds are currently being examined for the presence of functionally active sperm producing cells.

Egg production and egg quality traits

Birds on the Sel-Plex® diet entered egg production slightly behind the other feeding treatments (non-significant difference), but caught up within a few weeks. Early egg production to 29 wk of age was not different (Table 4). In fact, the rate of lay was similar through most of the production period. However, during the late lay period (49-58 weeks) the hen-housed rate of lay was 68% in Sel-Plex® birds compared to 61% and 60% in the selenite and non-supplemented treatments, respectively. The Sel-Plex® birds produced an extra 5 eggs/bird during this period, on average. This is an important time to be producing more eggs, as egg size is higher than in
young breeders, which results in a larger chick size and ultimately a greater broiler weight. Edens (2002) also indicated that the egg production of Sel-Plex®-fed hens initially lagged behind, but caught up and even surpassed that of the selenite-fed hens after 5 wk.

Ultimately the settable egg production in the dietary groups was 168.5 (non-supplemented), 168.6 (selenite), and 174.6 (Sel-Plex®) eggs/bird (Table 4). Overall, unsettable egg production ranged from 3.49% in non-supplemented hens to 1.9% in Sel-Plex® hens and was not significantly different. However, during the late lay period (49-58 wk), the Sel-Plex® hens produced significantly fewer unsettable eggs (0.9%) than non-supplemented hens (3.3%), while selenite hens were intermediate (1.7%). Egg weight and shell quality of settable eggs was assessed throughout the trial and was unaffected. This means that if the hen laid a good egg, it also had a good shell. However, diet affected how many eggs were produced with good shells, as shell defects were the primary egg quality problem in unsettable eggs. Feeding Sel-Plex® organic selenium to laying hens at 80 wk of age has previously been shown to improve shell breaking strength (Paton et al., 2000a).

Interestingly, dietary selenium affected the change in shell weight as the hens aged. Between 36 and 56 wk of age, shell weight increased by 0.55, 0.80, and 0.76 g in eggs of the non-supplemented, selenite, and Sel-Plex®-fed hens, respectively. During this time egg size also increased, meaning that the shell was being stretched over more egg, and therefore making up a smaller percentage of total egg weight. The proportion of shell weight dropped by 0.84% of egg weight in non-supplemented hens, 0.80% in selenite-fed hens, and 0.57% in Sel-Plex® hens between 36 and 56 wk of age. The Sel-Plex® hens were significantly less affected by age-related declines in the proportion of egg shell than the non-supplemented hens. While egg specific gravity was not significantly affected, this may be an indicator of increased shell thickness in the Sel-Plex® treatment (not tested). If this were different, there could be implications for incubation success and for defense from contamination in the barn.

Hen body weight followed a similar pattern throughout the production period. However, the non-supplemented hens grew heavier than the other treatment hens by 42 weeks of age. This difference carried through to 58 weeks of age. This comparison is somewhat artificial, as the body weight profile of the non-supplemented group was inflated by hens that dropped out of lay at a fairly young age. Nutrients they were no longer allocating to egg production went into growth instead. By the end of the trial, 100% of the Sel-Plex® hens were still in active production (Table 4) with no effect on their body weight relative to that of hens on the other treatments.

### Table 4. The effect of dietary selenium level and source on egg production traits of broiler breeders.

<table>
<thead>
<tr>
<th>Hen-housed egg production (%)</th>
<th>Settable egg production (%)</th>
<th>Total egg production (No.)</th>
<th>Unsettable egg production (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-28 wk</td>
<td>29-38 wk</td>
<td>39-48 wk</td>
<td>49-58 wk</td>
</tr>
<tr>
<td>No added Se</td>
<td>48.9</td>
<td>88.9</td>
<td>75.6</td>
</tr>
<tr>
<td>Selenite</td>
<td>49.7</td>
<td>88.1</td>
<td>73.1</td>
</tr>
<tr>
<td>Sel-Plex®</td>
<td>46.4</td>
<td>87.7</td>
<td>75.5</td>
</tr>
</tbody>
</table>

\( a,b \) Means within a column with no common superscript differ (P<0.05).

\( 1 \) Includes double-yolked, soft-shelled, membranous, and abnormally-shelled eggs.

\( 2 \) 0.3 ppm Se

Fertility, hatchability, and embryonic mortality

Prior to 34 weeks, hatchability averaged 88% in Sel-Plex® treatment eggs compared to 80% in selenite-fed
Reproductive responses to Sel-Plex® organic selenium in broiler breeders

birds and 77% in non-supplemented birds, and was similar in all treatments after peak production. Overall, fertility, hatchability, and hatch-of-fertile eggs demonstrated the beneficial nature of dietary selenium, but did not differentiate between selenium sources (Table 5). Fertility, for example, was 86.9% in non-supplemented hens compared to 90.1% in hens on selenite and Sel-Plex® diets. Not including selenium in the diet did not seriously harm hatchability, which is in contrast with work by Latshaw and Osman (1974) demonstrating a drop in hatchability to 18% in selenium-deficient hens. The current study may have provided more naturally occurring selenium in the other feed ingredients and the non-supplemented dietary treatment was not imposed until photostimulation (22 weeks of age).

Embryonic mortality can be a telling identifier of specific dietary or genetic effects. Problems with early embryonic mortality (1-14 days of incubation) can point to nutrient deficiencies. In this study, 5.33% of non-supplemented embryos died during this period compared to 3.72% (selenite) and 3.52% (Sel-Plex®) in the selenium-supplemented hens (Table 5). While selenium source did not make a difference here, clearly selenium supplementation was shown to be important. A beneficial effect of organic selenium was expected for the late incubation and hatch period, as this is the time of the greatest oxidative load for the embryo, and when the protective antioxidant effects of the Sel-Plex® may be most apparent. Variability among birds reduced the significance of this comparison, however, and late embryonic mortality stayed almost constant in non-supplemented and selenite hens, while it decreased in Sel-Plex® hens (Figure 1). As feed allocations were reduced with age, the micronutrients would have been in shorter supply. The improved efficiency of selenium uptake in the Sel-Plex® diet may not have made a substantial difference on hatchability until a nutrient challenge was faced by the flock. This fits with observations that Sel-Plex® can demonstrate benefits in stressful situations. Heat stress and long-term egg storage are examples of stress situations where Sel-Plex® has been shown to help. Surai and Dvorska (2001) indicate that there are numerous on-farm stress conditions that could be alleviated in part by organic selenium supplementation.

Ultimately what determines the success of a broiler breeder management program is chick production. In this trial, chick production was calculated from the hatchability of settable eggs. The unsupplemented hens produced an average of 131.3 chicks/hen-housed by 58 weeks of age, while selenite hens produced 139.1 chicks/hen, and Sel-Plex® hens produced 145.3 chicks/hen (Table 5). Between the selenite and Sel-Plex® selenium source diets, the numerical differences in settable eggs, embryonic mortality, hatchability, and hatch of fertile culminated in a difference of 5.8 chicks in favor of the Sel-Plex® hens. This difference increased to 14.1 chicks when compared to the non-supplemented hens.

Table 5. The effect of dietary selenium level and source on fertility, hatchability, embryo mortality and chick production traits of broiler breeder females.

<table>
<thead>
<tr>
<th></th>
<th>Embryo mortality and culls</th>
<th>Hatch of fertile ²</th>
<th>Chick production³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infertile (%)</td>
<td>Day 1-14 (%)</td>
<td>Day 15-hatch (%)</td>
</tr>
<tr>
<td>No added Se</td>
<td>13.06a</td>
<td>5.33a</td>
<td>3.66</td>
</tr>
<tr>
<td>Selenite⁴</td>
<td>9.91b</td>
<td>3.72b</td>
<td>3.85</td>
</tr>
<tr>
<td>Sel-Plex®⁴</td>
<td>9.87b</td>
<td>3.52b</td>
<td>3.14</td>
</tr>
</tbody>
</table>

a,b Means within a column with no common superscript differ (P<0.05).
¹Includes embryo mortality to hatch, dead-in-shell, and hatchery culls.
²Hatchability calculated only from fertile eggs set (infertile eggs excluded).
³Chick production = hatchability X settable eggs.
⁴0.3 ppm Se.
SUMMARY: STUDY 2

Reproductive traits were improved with the inclusion of dietary selenium, while Sel-Plex® supplementation also improved sperm survival in the oviduct, as well as settable egg production late in lay through increased egg production and reduced shell defects. Ultimately, chick production was improved in the Sel-Plex® treatment through more successful settable egg production and the additive culmination of numerical improvements in hatchability and embryo viability measurements. Selenium is essential in the diet for a successful reproductive effort. Additional benefits of using the Sel-Plex® are also possible. Selenium source appears to influence the hen’s contribution to the fertility of the breeder flock and to beneficially affect semen volume early in production.

Conclusions

Managing the broiler breeder female for optimal chick production requires an understanding of reproductive physiology, nutrition, and their interaction. Besides a thorough knowledge of everyday management, there must also be an awareness of feed ingredients and their interactions both with each other and with environmental effects. Whereas the basic composition of the egg is fairly constant, diet and specific feed ingredients can affect what and how much of some of the minor ingredients make it into the egg and ultimately the embryo. Specialized feed ingredients are available that behave differently than traditional ingredients and can enhance egg and chick quality under the right conditions. Together these factors can be used to enhance embryo survival.

References


Cantor, A.H. 1997. The role of selenium in poultry
selenium better for animals than inorganic sources? Two different scenarios in stress conditions. Feed Mix 9:8-10.


Introduction

Following recent reviews on the setting and the meeting of standards for the efficient replacement of pronutrient antibiotics in pig and poultry nutrition (Rosen, 2003b; 2003c), this review concentrates attention on ways and means of improving and optimizing the use of pronutrient antibiotics in broiler and turkey feeds, with particular reference to the multiplicity of proffered candidates and to the multiplexity and interactivity of influential genetic, environmental, managemental and dietary variables.

Why replace pronutrient antibiotics?

Contentiously but concretely, the search for and validation of antibiotic replacements stems from the attitudes and demands of consumers and their retail suppliers and from legislation based on precautionary principles. Soundly-based estimates of the effects on production costs and on retail prices are sparse. But the number of products offered as alternatives and the volume of literature thereon have risen steeply since 1999. Many hundreds, possibly thousands, of products are on offer. One can envisage several years’ work ahead to sort the wheat from the chaff.

Irrespective of questions of right or wrong in the proscription of prescription-free usage of antimicrobials in food production, the need to deploy replacements is expanding rapidly, creating a formidable task for scientists and producers in their search for fully-effective alternatives. The potential value of feed antibiotics was first demonstrated in the US by Moore et al. (1946) before commercialization in 1949. Potential problems caused by bacterial resistance phenomena were early sources of concern in human medicine, veterinary medicine and farm animal nutrition. In the early 1950s, opponents of antibiotic routines in poultry feeds initiated resistance research. Concerns were heightened by the Japanese discovery in 1959-1960 of infectious or transferable (as against natural) antibiotic resistance involving natural selection, mutation and DNA fragment transfer. The Swann Committee (1969) reported on the use of antibiotics in animal husbandry and veterinary medicine and initiated legislative restrictions banning the use of penicillin and tetracyclines without veterinary prescription. The European Economic Community followed this lead. The US Food and Drug Administration did not. In 1998, the European Union cancelled its approvals of six feed antibiotics, with intent to ban the remainder not later than 31 December, 2005.

Which are the candidates?

Table 1 contains a short list of the seven main categories of antibiotic replacement candidates. Whatever their nature or chemical composition, and no matter how numerous and multifarious are their known or hypothesized modes of action, the common thread in the present context resides in their abilities to enhance poultry performance at least as efficiently as the replaced pronutrient antibiotics in terms of feed conversion efficiency, mortality, liveweight, animal product yield and environmental depollution, measured integrally as net return on investment. The plethora of proffered candidates is evidenced, by way of example, in the 2000-01 Direct-Fed Microbial, Enzyme and Forage Additives Compendium (Miller
Optimizing the replacement of pronutrient antibiotics in poultry nutrition

Publishing Co.), by its lists of 222 candidate products, 90 enzymes, 72 microbials, 40 yeasts, 13 moulds and acids and seven oligosaccharides for use in poultry production alone.

Table 1. Major antibiotic replacements.

<table>
<thead>
<tr>
<th>Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticoccidials</td>
</tr>
<tr>
<td>Botanicals</td>
</tr>
<tr>
<td>Enzymes</td>
</tr>
<tr>
<td>Nutrients</td>
</tr>
<tr>
<td>Microbials</td>
</tr>
<tr>
<td>Oligosaccharides</td>
</tr>
</tbody>
</table>

Nutrients are also included because supplementary amino acids, minerals, purified energy sources and vitamins overlap performance-wise (Rosen, 1997) and may well overlap with pronutrients in nutritional improvements, irrespective of differences in modes of action.

Is nomenclature satisfactory?

More meaningful, standardized, transparent terminology in this field would benefit scientists, legislators and, above all, consumers. Feed additive antibiotics have been variously named as growth promoters, growth permiters, performance promoters, performance enhancers, production aids, feed economizers, nutrient balancers, efficiency enhancers, digestive enhancers and nutrition improvers, which descriptors have often been regarded in several senses as inaccurate, incomplete or inappropriate. Notwithstanding long-term, near-universal usage, the word ‘additive’ is nonetheless ill-conceived, being insufficiently descriptive. In practice the term ‘additive’ can generate auras of minority, subsidiarity, afterthought, optional extra and contaminant. It may be suitable for fuels, but not for food or feed; but it is doubtful whether legislators would ever countenance a change.

The term ‘pronutrient’ has been introduced to replace ‘additive’. A pronutrient is defined as a substance that improves the value of nutrients. As anticipated, a survey of 100 consumers has confirmed it as unknown to them, but 85 interpreted it as ‘something good for nutrition’. Therefore it is suggested that pronutrient could usefully replace the term “nonnutrient additive” used by the National Research Council to distinguish an additive from a nutrient. However, “nonnutrient additive” is unsatisfactory because it fails to convey the nutritional benefits of additives, which can be tantamount to those of nutrients.

The role of a pronutrient is portrayed in the schema in Figure 1. This shows the functional relationship of a pronutrient in nutrition relative to that of an antinutrient.

Figure 1. Effects of pronutrients and antinutrients in diets.

Pronutrients function via a hundred or more different modes of action in extending the value of the limiting nutrient in a diet. There is thus an overlap between the provision of a pronutrient and a ‘topping-up’ of a limiting nutrient. Hence, nutrients are, as aforementioned, included as a Table 1 category for consideration herein. The choice of a pronutrient or a nutrient in replacing feed antibiotics is simply a question of relative cost-effectiveness.

The terms ‘probiotic’ and ‘prebiotic’ are both plagiarisms, appropriated by scientists and suppliers. The US Food and Drug Administration and the European Union Commission, as regulatory authorities, both refused them as vagaries, opting respectively for direct-fed ‘microbial’ and ‘microorganism’. The term probiotic was, as a matter of fact, first coined by Winter (1955) in his research on botanical antibiotics found in cruciferous plants, when he stated that ‘we can call these substances probiotics; they are antibiotic against pathogenic microbes and they are therefore probiotic for the infected organism’. Gibson and Roberfroid (1994) took the ‘prebiotic concept’ from its original and literally-correct meaning and long-standing usage to define a chemical involved in the origin of life. Prebiotic is incorrect as an appellation for a
biosynthesized molecule, which is biofunctional, which nourishes a live microorganism and which inhabits a live host, in no sense whatsoever, before life.

The adoption of simple, realistic duplex descriptors to simultaneously impart nature and function could be a useful forward step in this field. For example, we could specify (a) pronutrient cellulase, formate, *B. cereus*, capisum, etc.; (b) prophylactic narasin, nifursol, *P. acidilactici*, mushroom polysaccharide, etc., (c) therapeutic tylosin phosphate, lincomycin hydrochloride, penicillin, trimethoprim, etc. and (d) pro-environmental phytase, carbohydrase, protease, etc. Furthermore, it would be advantageous to discontinue reference to ‘antibiotic alternatives’ or ‘antibiotic substitutes’, thereby avoiding a rekindling of consumers’ scientifically-unproven, anti-antibiotic prejudices.

**How are antibiotic replacements compared?**

All available properly-controlled test data need to be taken into account. The use of a handful of tests can illustrate the potential of a product as a starting point, but five tests cannot take account of the wide range of genetic, environmental, managemental and dietary factors affecting response in praxis. A recent survey on the current status and future needs of replacements based on the view of 50 suppliers, users, consultants, educators, communicators and academics revealed a large number of problems met in comparing candidate efficacies (Rosen, 2003c). Of the 92 nominated, the main problem areas are variation in response, use of uncontrolled tests, inadequate test designs, missing feed compositions, invalid ‘field’ tests and unjustified dosage recommendations.

Serious shortcomings in commonly-used averaging procedures are illustrated in Table 2. For example, the comparison of the effects on feed conversion ratio (FCReff) of enzymes and antibiotics, as such or in percentage terms, cannot be meaningful due to their 11-day mean duration difference. It is known that FCReff diminishes through starter to finisher phases. The same applies to time span (year) differences, averaging 1972 and 1987. The coefficients of variation in response of 129-1,449% constitute a warning against comparisons based on small test numbers.

For some researchers, direct comparisons within tests are fundamental, but they are, more often than not, too expensive. They require much larger, more costly experiments to manifest statistically significant differences between pairs of candidates, which differences are normally much smaller than those between candidates and negative controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Antibiotics</th>
<th>Enzymes</th>
<th>Microbials</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>5,159/1,521</td>
<td>2,557/439</td>
<td>234/57</td>
</tr>
<tr>
<td>FDIC, g</td>
<td>2.478</td>
<td>2.106</td>
<td>2.636</td>
</tr>
<tr>
<td>FDICeff, g</td>
<td>15.0 (0.6%)</td>
<td>32.4 (1.5%)</td>
<td>6.0 (0.2%)</td>
</tr>
<tr>
<td>CV, %</td>
<td>970</td>
<td>451</td>
<td>1,449</td>
</tr>
<tr>
<td>LWGC, g</td>
<td>1.075</td>
<td>1.043</td>
<td>1.331</td>
</tr>
<tr>
<td>LWGeff, g</td>
<td>39.8 (3.7%)</td>
<td>54.3 (5.2%)</td>
<td>25.3 (1.9%)</td>
</tr>
<tr>
<td>CV, %</td>
<td>129</td>
<td>147</td>
<td>192</td>
</tr>
<tr>
<td>FCRC</td>
<td>2.16</td>
<td>1.99</td>
<td>1.87</td>
</tr>
<tr>
<td>FCReff</td>
<td>-0.073 (-3.4%)</td>
<td>-0.105 (-5.3%)</td>
<td>-0.030 (-1.6%)</td>
</tr>
<tr>
<td>CV, %</td>
<td>164</td>
<td>185</td>
<td>195</td>
</tr>
<tr>
<td>MORTC, %</td>
<td>5.77</td>
<td>6.53</td>
<td>3.76</td>
</tr>
<tr>
<td>MORTeff, %</td>
<td>-1.40 (-24%)</td>
<td>-1.71 (-26%)</td>
<td>-0.40 (-11%)</td>
</tr>
<tr>
<td>CV, %</td>
<td>535</td>
<td>377</td>
<td>360</td>
</tr>
<tr>
<td>DUR*, days</td>
<td>41.0</td>
<td>30.3</td>
<td>35.8</td>
</tr>
<tr>
<td>YEAR – 1900</td>
<td>71.6</td>
<td>87.0</td>
<td>86.6</td>
</tr>
<tr>
<td>Improvement frequency², %</td>
<td>74</td>
<td>75</td>
<td>70</td>
</tr>
</tbody>
</table>

¹Mortality.
²Percentage of tests with improved FCReff and LWGeff.
*DUR = duration of test.
Comparative tests versus a positive control alone are contraindicated because they provide no evidence of a beneficial response to either candidate. Table 2 also starkly indicates a crucial gap in the large majority of tests, which fail to report mortality, viz. 71%, 83% and 76%, respectively for antibiotics, enzymes and microbials. Interestingly, the response improvement frequencies for these three candidate categories are virtually equal in the range 70-75%. Frequencies of beneficial response and magnitudes of variation therein merit greater attention than hitherto.

**How should we determine efficacy?**

In the light of the aforementioned shortcomings of few tests and superficial averaging, the answer to this question resides in the formulation of optimal multifactorial empirical algebraic models for nutritional effects of each candidate. Such models are used to calculate a requirement for any given set of circumstances and conditions, in order to compare an antibiotic and a candidate or to compare two or more candidates. Essentially, all published data are accepted. Manifestly-uncontrolled tests are excluded. Computer filing of all relevant data for the hundred or more potentially-important variables includes routines for elimination of errors in the original reports or in data abstraction and repeats. Performance must be measured from start to finish. If second or later phase data are required they are obtained, e.g., by subtracting 0-21 day from 0-42 day values. Standard statistical packages, e.g. Nie *et al.* (1975) and multiple regression methodology, e.g. Draper and Smith (1981) are used to determine best-fit models.

Best-fit algebraic models have statistically significant regressions, maximum multiple correlation coefficient squares, minimum standard deviations about regression and significant partial regression coefficients for all independent variables. After exclusion of aberrant (>3 x SD) responses, the emergent models are used to estimate nutritional responses and 95% confidence limits for any required set of values of the component independent variables. Differences in these predicted responses are tested for statistical significance. Nutritional response estimates are then used to compute and compare net profits to target liveweight and/or target duration.

These procedures are then used in feed formulation to quantify specific requirements for pronutrients or nutrient counterparts for target production. Thus one can assess whether a nutrient, e.g. L-methionine, would be a better choice than a pronutrient, e.g. endoxylanase or *Bacillus subtilis*. In other words, should one top up a limiting nutrient *per se* or increase the amount of the dietary limiting nutrient?

**Which are key variables?**

The 48 factors listed in Table 3 exemplify the range of variables potentially relevant in the elaboration of working models. There are also subsets of these factors to be considered. Routinely dose is considered as linear, quadratic, logarithmic or exponential. For broilers there are four sex types: male, female, mixed (50/50) and as-hatched. Ten to 20 antibiotics and five to 10 anticoccidials may be relevant, alone or in admixtures. Different disease challenges can be identified. The data emanate from more than 100 countries, though 10 or so usually furnish most of the test data. A few individual brands have numbers worthy of test. Types of oils and fats, animal protein, and vegetable proteins, including admixtures, total

<table>
<thead>
<tr>
<th>Control performance</th>
<th>Feed process</th>
<th>Maize²</th>
<th>Gross energy³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration</td>
<td>Antibiotic</td>
<td>Sorghum</td>
<td>Net energy³</td>
</tr>
<tr>
<td>Year of test</td>
<td>Anticoccidial</td>
<td>Wheat</td>
<td>Crude protein</td>
</tr>
<tr>
<td>Dose</td>
<td>Antihistominal</td>
<td>Barley</td>
<td>Crude fat</td>
</tr>
<tr>
<td>Initial age</td>
<td>Metabolic test</td>
<td>Oats</td>
<td>Crude fibre</td>
</tr>
<tr>
<td>Not day-old</td>
<td>Diet marker</td>
<td>Rye</td>
<td>Calcium</td>
</tr>
<tr>
<td>Sex</td>
<td>Part-purified diet</td>
<td>Animal fat</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>Phased dose</td>
<td>Disease challenge</td>
<td>Vegetable oil</td>
<td>Lysine</td>
</tr>
<tr>
<td>Factor 2 dose¹</td>
<td>Favson</td>
<td>Animal protein</td>
<td>Methionine</td>
</tr>
<tr>
<td>Selected birds</td>
<td>Institute test</td>
<td>Vegetable protein</td>
<td>Methionine + cystine</td>
</tr>
<tr>
<td>Housing</td>
<td>Country</td>
<td>Wheat offal</td>
<td>Threonine</td>
</tr>
<tr>
<td>Stocking density</td>
<td>Brand</td>
<td>Rice bran</td>
<td>Tryptophan</td>
</tr>
</tbody>
</table>

¹Second antibiotic/enzyme/acid/microbial/other pronutrient/nutrient
²Dietary concentrations (columns 3 and 4)
³Digestible or metabolizable energy are alternatives.
more than 200. For example, in Brozyme (Rosen, 2000; 2002) there are, including mixtures of each type, 71 animal proteins, 15 vegetable proteins, 81 oils and fats and 25 carbohydrate sources.

Based on experience to date, level of negative control performance, duration of feeding and year of test are basic, accounting for 10-50% of variation in effects. Contrastingly, some highly statistically significant factors can account for 1% or less. Interactions as product terms are normally relatively small contributors. In models containing 10 or more significant variables, dosage tends to be less important, often accounting for 7.5% or less of total variation.

Some independent variables are vital for the assessment of praxis values, compared with research conditions, namely, feed form, use of not-day-olds, males, cages, part-purified diets and specific disease challenges.

If the treated value is used as the dependent variable in modelling instead of the effect, multiple correlation coefficient squares (R²) are grossly inflated by the high correlation of control values and effects, e.g. for virginiamycin models (n=306), LWGeff and FCReff R² values of 0.14 and 0.44 respectively, become 0.97 for LWG and 0.99 for FCR as treated dependent variables. Such inflation of R² is misleading in variation accountancy and for some it harbours a delusion that R² less than 0.4-0.6 is unworthy.

Incomplete test reports limit the accountancy of variation. Recent data on phytase responses in broilers offer a useful perspective in which models containing 20 significant independent variables account for 64-72% of the sizeable variations in nutritional responses with time and place (Rosen, 2003a). Further progress, however, is unlikely until editors of peer-reviewed journals take a lead, for others to follow, in ensuring routine publication of fundamental variables such as temperature, altitude, lighting pattern, disease status and mortalities.

As examples, Figure 2 contains basal models for a group of five important broiler antibiotics in order to illustrate the magnitudes of the effects of key variables, with algebraic signs in accordance with nutrition science and practice.

These models contain 19 significant independent variables. For LWGeff and FCReff, negative coefficients quantify inferior and superior respective response contributions, with increase in LGWC and FCRC. Their positive DURs afford better and worse effects respectively with age increase. Diagnosed or endemic disease enhances LWGeff and FCReff. The partial regression coefficients of LGWC and FCRC mean that each 100 g better control performance would reduce LWGeff by 1.1 g and that each 10 point lower value for FCRC would reduce FCReff by 1.6 points.

How many tests are needed for a working model?

Hitherto, the notional n = c. 50 controlled tests has been thought of as a minimum required to produce a

![Figure 2.](image-url)
useful model to quantify the magnitudes of nutritional responses in feed formulation. A recent project addressed the question of a minimum platform via random fragmentation of a large 1,709/mortality 708 test resource of five of the most important feed antibiotics (i.e. two bacitracins, two tetracyclines and virginiamycin) into smaller subsets down to 34-43 for the elaboration of progressively smaller-based models (Rosen, 2004). The parent models are those in Figure 2. The analysis of a total of 704 fragmented resource models revealed that (a) no model at all was afforded in 23% of the random fragments; (b) progressive subset fractionation of the data set sharply reduced the number of significant variables from 79% in the parent models to 17% in the smallest, which averaged 1.2 significant independent variables; (c) the chance of three or more significant variables in subset models was one in eight; and (d) the smallest sets, averaging n = 38/mortality 15, did in toto reveal all the significant variables, albeit very patchily, found in the parent models.

How are requirement models used?

Requirements are needed for each and every set of circumstances for a defined target objective, for nutrients per se or for pronutrients. In other terms, what response might we expect within what confidence limits for a given dosage of supplementary methionine compared with an optimal dosage of bacitracin methylene disalicylate, or 6-phytase or a mannan oligosaccharide, etc.? The conjoint (overlap) consideration of the value of a limiting nutrient supplement or the improved efficiency of a limited nutrient supply with a pronutrient is of particular interest for the comparison of multifunctional substances. This approach could well resolve the ongoing, decades-old dispute on the relativities of DL-methionine (DLM) and methionine hydroxy-analogues (HMTBA and CaHMTB). The use of requirement models based on all-available, unselected control test data for DLM, HMTBA and CaHMTB with dose expressed simply as product weight (not molar or other equivalencies in malnutrition tests) should be decisive. Such methodology is imperative because DLM is a mixture of a nutrient and a prenutrient and HMTBA and CaHMTB are bifunctional as prenutrient and pronutrient.

The mode of application and value of multifactorial models for antibiotics and their replacements can next be illustrated in four examples as follows.

Using zinc bacitracin models based on 1,164 tests and first-generation phytase models (n=296), Table 4 shows that zinc bacitracin at 80 ppm affords a five point conversion improvement compared with the iso-cost level of phytase with no effect. In addition, a huge increase in phytase dosage (x 20) gives a three point conversion improvement, for which 42 ppm of a zinc bacitracin would suffice.

Table 4. Iso-input cost and iso-feed conversion effect comparisons of zinc bacitracin (n=1164) and first-generation phytases (n=296) for as-hatched 56 day-old broilers of LWGc=3,266g and FCRC=2.079.

<table>
<thead>
<tr>
<th>Pronutrient</th>
<th>Dosage (ppm)</th>
<th>Basis</th>
<th>LWGeff</th>
<th>FCReff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc bacitracin</td>
<td>80</td>
<td>iso-cost</td>
<td>59</td>
<td>-0.054</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>iso-FCReff</td>
<td>47</td>
<td>-0.032</td>
</tr>
<tr>
<td>Phytase (u/kg)</td>
<td>500</td>
<td>iso-cost</td>
<td>-1</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>10,000</td>
<td>iso-FCReff</td>
<td>159</td>
<td>-0.032</td>
</tr>
</tbody>
</table>

Secondly, Table 5 provides a model-based perspective on the results of a test from 7 to 28 days of age on female broiler chicks, which compared a Chinese herbal preparation, virginiamycin and a negative control (Guo et al., 2000). The herbal at 938 ppm afforded an FCReff four points inferior to virginiamycin at 20 ppm, even though the latter manifested, in this test, a response three points below its predicted result.

Table 5. Application of LWGeff and FCReff models to assess the results of a comparative test on a Chinese herbal formulation vs virginiamycin for LWGc=1,098 g and FCRC=1.554 on 7-28 day-old female birds.

<table>
<thead>
<tr>
<th>Product</th>
<th>Dosage (ppm)</th>
<th>Basis</th>
<th>LWGeff</th>
<th>FCReff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese herbal</td>
<td>938</td>
<td>tested</td>
<td>+15</td>
<td>+0.012</td>
</tr>
<tr>
<td>Virginiamycin</td>
<td>20</td>
<td>tested</td>
<td>-6</td>
<td>-0.027</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>predicted*</td>
<td>+3</td>
<td>-0.056</td>
</tr>
</tbody>
</table>

*306-test requirement models

The import and value of a negative control is further evidenced in a 39-day broiler test by Messikommer on 50 ppm zinc bacitracin versus a rhubarb supplement (Wenk, 2000). The predicted zinc bacitracin response of -0.074 ± 0.026 illustrates that the observed value of -0.049 is as expected; and that the botanical has potential value at 2,500 ppm (-0.038), but not at 5,000 ppm (+0.018).

Thirdly, truncated comparative tests without a negative control are preferred by some researchers, even though they are unable to confirm efficacy for either test product. An assessment of the results of a positive control only test can be made using a model.
for the test antibiotic. Syrvidis et al. (2003) reported a test on Digestarom 1317 Poultry Premium (a so-called phytobiotic) versus Flavomycin-80, but failed to include test dosages. Hence, a notional negative control performance of \( \text{LWG}_{\text{G}} = 2.125 \text{ g} \) and \( \text{FCR}_{\text{C}} = 1.970 \) was calculated from flavomycin models (\( n=394 \)) for a presumed dosage of 2 ppm. The computed negative control values reveal very large Digestarom responses of \( \text{LWG}_{\text{Geff}} = 326 \text{ g} \) (15.3%) and \( \text{FCR}_{\text{Reff}} = -0.220 \) (-11.2%). At today’s production standards, however, a feed conversion as-hatched for a 40-day 2,125 LWGC would be about 1.730, i.e., 12.2% less than the computed FCRC of 1.97 at 42 days old in this trial. Such a result against a positive control alone should be treated with reserve. The availability of further tests against negative controls would afford a better view on the potential of Digestarom. The same applies to any product if it claims value from one or more tests solely against a positive control(s).

Fourthly, even prior to the availability of working models for individual product brands of replacements, test data collections of the latter can be reviewed as to their potential value as antibiotic replacements. Bio-Mos® mannan oligosaccharide collections of 34 tests for broilers (Hooge, 2003a) and 27 tests for turkeys (Hooge, 2003b) are used to provide an example. Table 6 compares mean liveweight gain and feed conversion responses in broilers and turkeys with corresponding predictions for equivalent circumstances, using comprehensive multifactorial models for the antibiotic products virginiamycin (\( n=306 \)) in broilers and zinc bacitracin (\( n=226 \)) in turkeys.

Table 6. Comparison of mean LWGeff and FCReff in 34 mannan oligosaccharide (Bio-Mos®) broiler tests with predicted responses for virginiamycin for LWGC=2,149 g, FCRC=1.879 at 42.2 days-old and in 27 turkey tests with predicted responses for zinc bacitracin for LWGC=5,643 g, FCRC=1.981 at 68.7 days.

<table>
<thead>
<tr>
<th>Species/ product</th>
<th>Dosage</th>
<th>Basis</th>
<th>LWGeff (g)</th>
<th>FCReff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broilers</td>
<td>Bio-Mos®</td>
<td>1.04 g/kg</td>
<td>34 test mean +40</td>
<td>-0.042</td>
</tr>
<tr>
<td></td>
<td>Virginiamycin</td>
<td>20 ppm</td>
<td>305 test model +21</td>
<td>-0.040</td>
</tr>
<tr>
<td>Turkey</td>
<td>Bio-Mos®</td>
<td>1.19 g/kg</td>
<td>27 test mean +127</td>
<td>-0.032</td>
</tr>
<tr>
<td></td>
<td>Zn bacitracin</td>
<td>50 ppm</td>
<td>236 test model +148</td>
<td>-0.041</td>
</tr>
</tbody>
</table>

The Table 6 data are encouraging for Bio-Mos® as a replacement when used at a dosage of 1 g/kg in broiler and turkey feed. More searching comparisons, however, must now await the availability of requirement models for Bio-Mos®, quantifying the influences of dosage, level of bird performance, presence or absence of other pronutrients, especially anticoccidials, bird sex, feed form and limiting nutrients.

By virtue of extensive 15-year test programmes, exogenous enzymes would appear at present to be the best characterized replacement category. The data available for organic acids in pigs, microbials and oligosaccharides in poultry and pigs may already suffice for the elaboration of initial working models. But all available data should be used to avoid possible selection bias, as in the organic acid studies of Partanen and Mroz (1999) and Partanen (2001).

### How should we test admixtures?

Problems in the use of admixtures of antibiotic replacements arise from shortcomings in nomenclature and posology (dosage science). ‘Additivity’ and ‘synergism’ are often misused, usually for sub-additivity. The possibility of antagonism should always be borne in mind. For purposes of definition, the possible effects of admixture are classified herein as sub-additive, additive, synergistic, ineffective or antagonistic, as defined for the admixture of 2+3 of A and B providing 4, 5, 6, 2 or 3 and 1 unit(s) of response respectively. A 2 x 2 factorial test is a good starting point, shown in Table 7, which also includes other iso-cost admixtures for A or B alone or for higher single dosages of A or B. The acid test for maximal results uses A+B each at its economic optimum.

Table 7. Scope of admixture tests.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Feed concentration (ppm)</th>
<th>Investment cost (MU*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal feed</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Replacement A</td>
<td>a</td>
<td>4</td>
</tr>
<tr>
<td>Replacement B</td>
<td>b</td>
<td>8</td>
</tr>
<tr>
<td>Replacement A + Replacement B</td>
<td>a + b</td>
<td>12</td>
</tr>
<tr>
<td>Replacement A</td>
<td>3a</td>
<td>12</td>
</tr>
<tr>
<td>Replacement B</td>
<td>1.5b</td>
<td>12</td>
</tr>
<tr>
<td>Replacement A + Replacement B</td>
<td>0.33a + 0.33b</td>
<td>4</td>
</tr>
<tr>
<td>Replacement A + Replacement B</td>
<td>0.67a + 0.67b</td>
<td>8</td>
</tr>
</tbody>
</table>

*MU money units

Nutritional models can provide a useful guide towards maximum admixture efficiency by pre-determination of the economic optimal doses of Replacements A
and B. These admixture guidelines are also pertinent in situations where pronutrient antibiotics can still be utilized in admixture with nutrients or pronutrient supplements.

Quo vadimus?

The thesis presented herein essentially advocates that we should optimize the choice and dosage of nutrient or pronutrient antibiotic replacements by taking cognizance of all available data expressed in predictive, empirically-based, multifactorial multiple regression requirement models for feed, gain, conversion and mortality effects, at least. Such models should be updated annually to take account of the accelerating current flow of scientifically-controlled feeding tests, as seen for example, in the increase of exogenous enzyme publications from 1,422 up to the year 2000 rising to its latest content in the Brozyme resource (Rosen, 2002) of 2,175. It is intended also to extend these studies to table egg and breeder hens and at least ducks among the minor species.

Following the lead of this Symposium in ‘re-imagining the feed industry’, should we not also prune and improve its verbiage to better its science and raise its transparency to consumers, setting a good example for all members of the food chain?

In conclusion, it may be apposite, in line with the question and answer format of this review, to conclude with a Seven Question Test with which the user of an antibiotic replacement can assess the potential value of a supplier’s Product X.

1) How many properly-controlled feeding tests do you have on the efficacy of Product X?
2) How many of these have no negative controls?
3) Can you supply a bibliography for 1) and 2)?
4) How many times out of ten does Product X improve liveweight gain and feed conversion?
5) What are the coefficients of variation in the gain and conversion responses?
6) What dosage of Product X will maximize return on my investment and why?
7) Can you supply me with a model to predict responses to Product X under my conditions?

Receipt of answers of 1) 30; 2) five; 3) yes; 4) seven; 5) 100-200%; and 6) “x ppm because . . .” should be encouraging. A ‘yes’ to 7) would be even better.

References


Comparative aspects of *Fusarium* mycotoxicoses in broiler chickens, laying hens and turkeys and the efficacy of a polymeric glucomannan mycotoxin adsorbent: Mycosorb®

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**Introduction**

*Fusarium* fungi thrive in temperate climates around the world and *Fusarium* mycotoxins are the mycotoxins most commonly found in feed grains and forages (Wood, 1992). There are numerous pathologies characteristic of *Fusarium* mycotoxicoses and this is due to the several chemically distinct groups of *Fusarium* mycotoxins, which have very different effects on animal metabolism and behavior. Swine are usually considered to be the most sensitive species to feed-borne mycotoxins both with respect to feed refusal and to reproductive failure. Ruminant animals are thought to be the most resistant because of the detoxifying effect of rumen microorganisms. Poultry are generally considered to be less sensitive than swine and are often the recipients of contaminated grains diverted from swine feeds.

The *Fusarium* mycotoxin most often detected in Canadian-grown grains is the trichothecene deoxynivalenol (DON, vomitoxin) (Scott, 1997). Trichothecene toxicosis is characterized by reduced appetite, lesions of the intestinal tract and immunosuppression. Another frequently detected compound is zearalenone, an estrogenic mycotoxin that impairs mammalian reproduction.

It has been reported that broiler chicks can tolerate up to 15 ppm DON from naturally-contaminated wheat and oats without adverse effects on performance (Hulan and Proudfoot, 1982; Kubena *et al.*, 1987). Others, however, observed reduced performance and changes in immune function, hematology and serum chemistry in broiler chicks fed 16 to 18 ppm DON from naturally contaminated sources (Huff *et al.*, 1986; Kubena *et al.*, 1988; 1989; Harvey *et al.*, 1991). It is clear however, that broiler chickens are far less sensitive than swine to DON-contaminated feeds, particularly with respect to reduced feed intake (Smith *et al.*, 1997).

It has been reported that the feeding of 100 ppm zearalenone had no effect on layer performance, fertility or hatchability (Marks and Bacon, 1976). The feeding of DON at 5 ppm (Hamilton *et al.*, 1985) or 18 ppm (Kubena *et al.*, 1987) did not affect layer performance. Adverse effects on layer performance are often greater under field conditions (Keshavarz, 1993). It is often difficult, however, to explain why this occurs (Williams *et al.*, 1992).

There is less information available regarding the feeding of contaminated grains to turkeys. Hamilton *et al.* (1985) indicated that turkey poults can tolerate diets containing at least 5 ppm DON. Manley *et al.* (1988) described feed refusal and high mortality in a commercial turkey flock fed diets containing 81 ppb DON + 2.2 ppm salinomycin. The feeding of 4.4 ppm DON + 22 ppm salinomycin had no effect on feed consumption or viability. Morris *et al.* (1999) observed that the feeding of 20 ppm DON had no adverse effects on poults and no toxicological interaction was observed between DON and moniliformin.

The discrepancy between the relative tolerance of poultry to *Fusarium* mycotoxins in literature reports and the seeming susceptibility of poultry under commercial conditions may be due to several factors. Many of the experiments conducted in literature
reports were for fairly short periods. This is often necessary to conserve valuable stocks of purified mycotoxins or fungal culture materials. The use of purified mycotoxins, fungal culture materials or artificially inoculated corn can also bias findings because the toxicological synergism arising from the feeding of combinations of mycotoxins does not take place.

Polymeric mycotoxin adsorbents prevent mycotoxicoses by adsorbing mycotoxins in the intestinal lumen and preventing transfer through the blood to target tissues (Ramos et al., 1996). The current studies were conducted to determine the efficacy of Mycosorb®, a glucomannan polymer extracted from the cell wall of yeast, in preventing the adverse effects of blends of grains naturally-contaminated with Fusarium mycotoxins on poultry.

Materials and methods

EXPERIMENTAL FEEDSTUFFS

Mycotoxins in the current experiments were provided by a blend of naturally contaminated corn and wheat purchased from producers in Southwestern Ontario. The complete diets were analyzed for DON, 3-acetyl-DON, 15-acetyl-DON, nivalenol, T-2 toxin, iso T-2 toxin, acetyl-T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, fusarenon-X, diacetoxyscirpenol, scirpentriol, 15-acetoxyscirpentriol, neosolaniol, zearalenone, zearalenol, aflatoxin and fumonisin by gas chromatography and mass spectrometry at the Veterinary Diagnostic Laboratory, North Dakota State University, Fargo, North Dakota (Raymond et al., 2003). Fusaric acid was determined by the high performance liquid chromatographic method of Matsui and Watanabe (1988) as modified by Smith and Sousadias (1993) and confirmed by Porter et al. (1995).

EXPERIMENTAL DESIGN

Broilers

Broiler chicks were fed starter (0 – 3 weeks), grower (3 – 6 weeks) and finisher (6 – 8 weeks) diets in two 56-day experiments (Swamy et al., 2002; 2004a). Diets included: 1) control, 2) low level of contaminated grains, 3) high level of contaminated grains, and 4) high level of contaminated grains + 0.2% Mycosorb®. Growth rates and feed consumption were monitored weekly. At the end of the study, blood and tissue samples were taken for hematology and serum chemistry measurements.

Laying hens

One hundred and forty-four, 45-week-old laying hens were fed for 12 weeks diets including: 1) control, 2) contaminated grains, and 3) contaminated grains + 0.2% Mycosorb®. Parameters measured included feed consumption, egg production, egg shell measurements, egg quality measurements, relative organ weights and plasma chemistry.

Turkeys

Two hundred and twenty-five day-old male turkey pouls were fed corn, wheat and soybean meal-based starter (0–3 weeks), grower (3–6 weeks), developer (6 – 9 weeks) and finisher (10 – 12 weeks) diets in a 12-week experiment. Diets included: 1) control, 2) contaminated grains, and 3) contaminated grains + 0.2% Mycosorb®. Parameters measured included weight gain, feed consumption, relative organ weights and plasma chemistry.

Results

MYCOTOXIN ANALYSES

Of the twenty different mycotoxins analyzed for, only DON, 15-acetyl DON, zearalenone and fusaric acid were found in detectable quantities in all experimental diets. DON was found to be approximately 0.5 ppm, 5.0 ppm, 9.0 ppm and 10.0 ppm for the four experimental broiler diets. The concentrations of 15-acetyl DON were about 0.5 ppm, zearalenone was about 0.5 ppm and fusaric acid was about 17 ppm. Diets fed to laying hens which contained contaminated grains had an average of 12.0 ppm DON with the other three mycotoxins in the same ratios as were seen in the broiler trials. In the turkey trial, diets containing contaminated grains averaged 6.5 ppm, 7.6 ppm, 10.6 ppm and 13.3 ppm DON for the starter, grower, developer and finisher phases with the other three mycotoxins present in the same proportions as were seen for the broiler and layer experiments.

BROILERS

There was a significant linear decrease in growth rate and feed consumption in the grower period when
increasing levels of contaminated grains were fed to rapidly growing broilers (Swamy et al., 2004a; Table 1). No significant effect of diet was seen in the starter or finisher periods. When broilers were growing more slowly, growth depression was observed in the finisher phase (Swamy et al., 2002). At the end of the finisher phase in the earlier study, it was observed that the feeding of contaminated grains elevated red blood cell count and blood concentrations of hemoglobin and uric acid (Table 2). Biliary concentrations of immunoglobulin A were reduced while breast meat redness increased.

The feeding of 0.2% Mycosorb® prevented all of the above dietary effects.

LAYING HENS

The feeding of contaminated grains decreased feed consumption compared to controls in the first month (P<0.05) (Table 3). Feed intake increased, however, in the second and third months. The feeding of Mycosorb® prevented this increase in the third month and also prevented a decrease in feed efficiency. At the end of the experiment, it was observed that hens fed contaminated grains had an increased relative kidney weight compared to controls; this effect was prevented by the feeding of Mycosorb®.

Egg production and egg mass decreased (P<0.05) compared to controls in the first two months when contaminated grains were fed. The feeding of Mycosorb® prevented this in the first month. The most obvious effect of feeding contaminated grains on plasma chemistry was on uric acid concentrations (Table 4). In each month, plasma uric acid concentrations were significantly increased with the feeding of contaminated grains. In each case, this increase was prevented by the feeding of Mycosorb®.

TURKEYS

The feeding of contaminated grains reduced growth rates in the starter, developer and finisher phases and overall (Table 5). The feeding of Mycosorb® prevented these effects. The most obvious effect of

---

### Table 1. Effect of feeding blends of grains naturally-contaminated with Fusarium mycotoxins on weight gain and feed consumption of broiler chickens.¹

<table>
<thead>
<tr>
<th>Diet</th>
<th>Feed consumption (g/bird)</th>
<th>Weight gain (g/bird)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 - 21 days</td>
<td>21 - 42 days</td>
</tr>
<tr>
<td>Control</td>
<td>908</td>
<td>2797</td>
</tr>
<tr>
<td>Low mycotoxins</td>
<td>841</td>
<td>2565</td>
</tr>
<tr>
<td>High mycotoxins</td>
<td>923</td>
<td>2392</td>
</tr>
<tr>
<td>High mycotoxins + 0.2% Mycosorb®</td>
<td>968</td>
<td>2472</td>
</tr>
<tr>
<td>SEM</td>
<td>37</td>
<td>60</td>
</tr>
<tr>
<td>Linear effect</td>
<td>NS¹</td>
<td>0.05</td>
</tr>
</tbody>
</table>

¹From Swamy et al., 2004a.
²Values are least square means; n = 3.
³Values are least square means; n = 90.
⁴Not significant (P>0.05).

### Table 2. Effect of feeding blends of grains naturally-contaminated with Fusarium mycotoxins on hematology, serum chemistry and breast meat coloration of broiler chickens.¹

<table>
<thead>
<tr>
<th>Diet</th>
<th>RBC²</th>
<th>Hb³</th>
<th>Uric acid⁴</th>
<th>Redness⁵</th>
<th>Biliary IgA²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.66</td>
<td>95.0</td>
<td>259</td>
<td>0.45</td>
<td>7.54</td>
</tr>
<tr>
<td>Low mycotoxins</td>
<td>2.84</td>
<td>101.1</td>
<td>286</td>
<td>0.67</td>
<td>7.28</td>
</tr>
<tr>
<td>High mycotoxins</td>
<td>2.83</td>
<td>99.2</td>
<td>357</td>
<td>0.80</td>
<td>4.99</td>
</tr>
<tr>
<td>High mycotoxins + 0.2% Mycosorb®</td>
<td>2.54</td>
<td>91.2</td>
<td>281</td>
<td>0.21</td>
<td>6.54</td>
</tr>
<tr>
<td>SEM</td>
<td>0.04</td>
<td>1.37</td>
<td>10.9</td>
<td>0.07</td>
<td>0.29</td>
</tr>
<tr>
<td>Linear effect</td>
<td>0.01</td>
<td>0.01</td>
<td>0.009</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

²Red blood corpuscle counts (10⁹/L); n = 12.
³Hemoglobin concentration (ppm); n = 12.
⁴Uric acid concentration (µmoles /L); n = 12.
⁵Unitless scale, 0 = green, 1 = red; n = 15.
⁶mm precipitate; n = 15.
Comparative aspects of Fusarium mycotoxicoses in poultry

Table 3. Effect of feeding blends of grains naturally-contaminated with Fusarium mycotoxins on feed consumption and feed efficiency of laying hens.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Feed consumption¹ (g/hd/day)</th>
<th>Feed efficiency² (feed/egg mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wk 0 - 4</td>
<td>Wk 4 - 8</td>
</tr>
<tr>
<td>Control</td>
<td>119</td>
<td>120</td>
</tr>
<tr>
<td>Mycotoxins</td>
<td>106</td>
<td>127</td>
</tr>
<tr>
<td>Mycotoxins + 0.2% Mycosorb®</td>
<td>114</td>
<td>124</td>
</tr>
<tr>
<td>Pooled SD</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Control vs mycotoxins</td>
<td>0.008</td>
<td>0.04</td>
</tr>
<tr>
<td>Mycotoxins vs Mycosorb®</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

¹n = 12.
²n = 12.
³Not significant (P>0.05).

Table 4. Effect of feeding blends of grains naturally-contaminated with Fusarium mycotoxins on organ weights and plasma uric acid concentrations of laying hens.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Liver (g)</th>
<th>Spleen (g)</th>
<th>Kidney (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wk 4</td>
<td>Wk 8</td>
<td>Wk 12</td>
</tr>
<tr>
<td>Control</td>
<td>44.5¹</td>
<td>2.2</td>
<td>5.9</td>
</tr>
<tr>
<td>Mycotoxins</td>
<td>44.2</td>
<td>2.4</td>
<td>7.6</td>
</tr>
<tr>
<td>Mycotoxins + 0.2% Mycosorb®</td>
<td>46.2</td>
<td>2.2</td>
<td>6.6</td>
</tr>
<tr>
<td>Pooled SD</td>
<td>7.58</td>
<td>0.66</td>
<td>0.98</td>
</tr>
<tr>
<td>Control vs mycotoxins</td>
<td>NS²</td>
<td>NS</td>
<td>0.002</td>
</tr>
<tr>
<td>Mycotoxins vs Mycosorb®</td>
<td>NS</td>
<td>NS</td>
<td>0.02</td>
</tr>
</tbody>
</table>

¹n = 12.
²Not significant (P>0.05).

Table 5. Effect of feeding blends of grains naturally-contaminated with Fusarium mycotoxins on growth of turkeys (g/bird/week).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Starter</th>
<th>Grower</th>
<th>Developer</th>
<th>Finisher</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>217</td>
<td>378</td>
<td>748</td>
<td>723</td>
<td>517</td>
</tr>
<tr>
<td>Mycotoxins</td>
<td>196</td>
<td>492</td>
<td>657</td>
<td>637</td>
<td>496</td>
</tr>
<tr>
<td>Mycotoxins + 0.2% Mycosorb®</td>
<td>213</td>
<td>548</td>
<td>756</td>
<td>852</td>
<td>592</td>
</tr>
<tr>
<td>Control vs mycotoxins</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Mycotoxins vs Mycosorb®</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

diet on plasma chemistry was on plasma uric acid concentrations (Table 6). After 4 and 8 weeks of feeding contaminated grains, plasma uric acid concentrations were significantly reduced. This effect was not seen, however, when birds received Mycosorb®. At the end of the experiment, turkeys fed contaminated grains + Mycosorb® had significantly smaller spleens and kidneys and significantly larger bursas than birds fed unsupplemented contaminated grains.

Table 6. Effect of feeding blends of grains naturally-contaminated with Fusarium mycotoxins on organ weights and plasma uric acid concentrations of turkeys.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Bursa (g)</th>
<th>Spleen (g)</th>
<th>Kidney (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wk 4</td>
<td>Wk 8</td>
<td>Wk 12</td>
</tr>
<tr>
<td>Control</td>
<td>5.3¹</td>
<td>5.6</td>
<td>16.1</td>
</tr>
<tr>
<td>Mycotoxins</td>
<td>5.5</td>
<td>6.1</td>
<td>17.7</td>
</tr>
<tr>
<td>Mycotoxins + 0.2% Mycosorb®</td>
<td>6.5</td>
<td>5.1</td>
<td>15.8</td>
</tr>
<tr>
<td>SEM</td>
<td>0.30</td>
<td>0.33</td>
<td>0.71</td>
</tr>
<tr>
<td>Control vs mycotoxins</td>
<td>NS²</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Mycotoxins vs Mycosorb®</td>
<td>0.03</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

¹n = 12.
²Not significant (P>0.05).
Discussion

Broilers, layers and turkeys are all sensitive to *Fusarium* mycotoxicoses. The adverse effects of the diets fed in the current studies are greater than literature reports based on the DON content. This is likely because of the relatively short duration of previously reported trials. The blending of different naturally contaminated grains, moreover, also results in a more complex mixture of mycotoxins thereby increasing the chances of toxicological synergy between mycotoxins.

BROILERS

The observation that feeding contaminated grains to broilers reduced growth only in the grower and finisher phases supports the concept that broilers do not exhibit feed refusal in a manner similar to swine fed *Fusarium* mycotoxin contaminated diets (Smith *et al*., 1997). The reason for this species difference has been shown to be differences in the effects on brain neurochemistry (Swamy *et al*., 2004b). The feeding of contaminated grains to pigs elevated brain serotonin concentrations. In broilers, such treatments elevated both serotonin and catecholamines thereby canceling the effect of serotonin on appetite suppression. It is likely that mycotoxin-induced growth suppression in broilers was due to gradual alterations in metabolism that occurred with extended feeding of contaminated grains.

The mycotoxin-induced elevation in red blood cell count and hemoglobin is similar to the changes seen in ascites. It is possible that the hypotensive effect of fusaric acid may be reducing blood flow to the lungs resulting in an increased need for oxygen trapping capacity of blood. Elevations in blood uric acid concentrations were likely due to the inhibition of protein synthesis caused by trichothecenes such as DON and 15-acetyl DON. This would result in increased hepatic oxidation of amino acids and increased uric acid excretion. Red discoloration of breast meat has been reported in turkeys fed *Fusarium* culture filtrates (Wu *et al*., 1994). The discoloration seen in the current study was likely due to increased red blood cell count and hemoglobin concentrations as well as to edema arising from the hypotensive effects of fusaric acid. The reduced biliary immunoglobulin A concentrations may have arisen from trichothecene-induced inhibition of protein synthesis.

LAYING HENS

The effect of feeding contaminated grains on feed intake of laying hens is in contrast to that seen in broilers. It would seem that after an initial reduction in feed intake and egg production, layers increased feed intake perhaps in an attempt to boost egg production. The result, however was a very dramatic deterioration in feed efficiency (feed consumed/egg mass). This may be due, in part, to increased hepatic amino acid oxidation due to the trichothecene-induced reduction in protein synthesis. It is notable that the mycotoxin-induced elevation in blood uric acid concentrations is similar to, but greater in magnitude than, the response seen in broilers. The increased kidney weight seen when contaminated grains were fed is likely due to the increased metabolic burden of excretion arising from increased uric acid synthesis.

TURKEYS

Feeding contaminated grains significantly reduced weight gain of turkey poults as early as the second week of feeding. This is a more rapid effect than was seen in broilers but little effect of diet was seen on feed consumption. It would appear that turkeys are more sensitive to this mycotoxin challenge than broilers. The significant depression in blood uric acid concentrations after 4 and 8 weeks of feeding is in marked contrast to the responses seen in broilers and laying hens. The metabolic reason for this remains to be determined as other indices of blood chemistry were largely unaffected by diet.

EFFECTS OF MYCOSORB®

Mycosorb® proved to be a very effective preventative treatment for *Fusarium* mycotoxicoses in broilers, layers and turkeys. The mode of action of Mycosorb® is to prevent intestinal uptake of mycotoxins and subsequent transfer of mycotoxins to sensitive target tissues such as liver, kidney, brain and reproductive tract. It is clear from the efficacy of Mycosorb® in these trials that it is capable of adsorbing a combination of *Fusarium* mycotoxins and minimizing the potential for toxicological synergism.
References


Swamy, H.V.L.N., T.K. Smith, P.F. Cotter, H.J.


Pig science
Creating technical and educational forums that help pig producers meet performance and economic goals: the Premier Pig Program™

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¹Close Consultancy, Wokingham, Berkshire, UK
²Alltech Inc., Melbourne, Victoria, Australia

Introduction

Regardless of where pigs are produced the objectives should be the same: to optimise the quantity of pig meat produced per sow per year or per lifetime, at minimal cost. For the modern sow and pig genotypes, with pigs being sold at 100 kg liveweight, the objective should be to produce at least 1 tonne of carcass lean per sow per year. This is achieved as in Table 1.

If pigs are sold at 120 kg body weight, then the target should be 1.25 tonne of carcass lean per sow per year, or 2.50 tonne carcass lean per lifetime. The performance of the animal at all stages of production, that is the sow, weaner and grow-finish pig, contribute to these target levels of performance, as indicated in Figure 1.

Table 1. Targets for producing 1 tonne of carcass lean per sow per year with pigs sold at 100 kg liveweight.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 kg liveweight at slaughter</td>
<td>75 kg carcass weight</td>
</tr>
<tr>
<td>60% lean in carcass</td>
<td>45 kg carcass lean</td>
</tr>
<tr>
<td>23 pigs sold/sow/year</td>
<td>1.03 tonne carcass lean per year</td>
</tr>
<tr>
<td>5 litters/sow/lifetime</td>
<td>2.06 tonne carcass lean per lifetime</td>
</tr>
</tbody>
</table>

Figure 1. Primary factors affecting pig performance.
Indeed, it is important to establish target levels of performance for each stage of production, so that producers can benchmark the performance on their own farm relative to industry standards. Suggested indices for the breeding, weaner and grow-finish animal are listed in Table 2.

If performance is below expectation, then it is important to identify those factors that limit performance and to take appropriate actions to avoid loss of productivity. This is the aim of the Premier Pig Program™.

**Objectives of the Premier Pig Program™**

The Premier Pig Program™ has been developed to provide independent technical information and support appropriate to all sectors of the pig industry worldwide. Although many factors influence financial performance, the key factor influencing the efficiency and cost of production is nutrition, with the cost of feed representing 60-70% of total production costs in many countries. Understanding the nutritional needs of the pig at all stages of growth and providing the correct diets and nutritional management is fundamental to efficient and profitable production.

The major objectives of the Premier Pig Program™ are therefore:

- To provide user-friendly technical information and support to all sectors of the industry
- To suggest target objectives for modern pig production
- To compare actual and target levels of productivity and identify areas of concern
- To propose actions and solutions that can be taken on-farm to enhance performance
- To provide technology to allow long-term sustainable production
- To help in the application of new concepts and technologies

A major feature of the program has been the publication of a manual, which details the appropriate nutrition and management of the pig at all stages of production. Information is provided on nutritional needs and on those factors that influence them. Differences in genetic potential, health status, housing and environmental conditions all influence animal performance and these must be considered, as well as the requirements of the market. Fortunately, our knowledge of the biology and nutritional physiology of the pig has advanced sufficiently in recent years to allow these to be considered in the development of any feeding and management strategies to meet individual needs and animal circumstances.

The manual also suggests target levels of performance so that producers can benchmark their farm’s performance against industry standards. It discusses what intervention strategies and actions can be taken on-farm if performance falls below expectation. In this way both production and economic efficiency can be improved.

The Premier Pig Program™ and the manual cover the following areas:

- Nutrients in the diet
- The breeding pig: sow and boar
- The weaner piglet
- The grower/finisher pig
- Meat quality
- Environmental issues

### Nutrients in the diet

The objective is to provide a reference base to explain the major roles of energy, protein, amino acids, minerals and vitamins for the normal functioning of the animal’s metabolism and for the maintenance of good levels of reproduction and growth, as well as its health and well being. Sources of nutrients are also discussed.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Target Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sow</td>
<td>24-25 piglets weaned per year</td>
</tr>
<tr>
<td>Weaned piglet</td>
<td>30 kg body weight at 10 weeks of age; Feed : gain &lt;1.5 : 1</td>
</tr>
<tr>
<td>Grow-finish pig</td>
<td>100 kg body weight at 21 weeks of age; Feed : gain &lt;2.5 : 1</td>
</tr>
<tr>
<td></td>
<td>120 kg body weight at 23 weeks of age; Feed : gain &lt;2.7 : 1</td>
</tr>
</tbody>
</table>

---

Table 2. Performance targets for sows, piglets and grow-finish pigs.
The breeding pig: sow and boar

The nutritional and management needs of the replacement gilt and the gestating and lactating sow are discussed, including those factors that influence them. This allows practical feeding and management strategies to be developed to optimise and to sustain long-term sow productivity.

Target levels of performance for the sow are suggested, as well as the threshold or intervention levels when action needs to be taken to avoid loss of productivity (Table 3). The nutritional, management and healthcare strategies needed to meet these target levels of productivity are discussed and the economic costs of lost productivity highlighted.

Table 3. Key factors influencing sow productivity.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Target</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culling rate, %</td>
<td>3.5</td>
<td>&gt;4.2</td>
</tr>
<tr>
<td>Sow parity at culling</td>
<td>6.7</td>
<td>&lt;3, &gt;8</td>
</tr>
<tr>
<td>Average parity</td>
<td>5.0</td>
<td>&lt;3, &gt;8</td>
</tr>
<tr>
<td>Sow mortality, %</td>
<td>&lt;5.0</td>
<td>&gt;5.0</td>
</tr>
<tr>
<td>Farrowing rate, %</td>
<td>90.0</td>
<td>&lt;83.0</td>
</tr>
<tr>
<td>Litters/sow/year</td>
<td>2.4</td>
<td>&lt;2.2</td>
</tr>
<tr>
<td>Wean-mating interval, days</td>
<td>5.0</td>
<td>&gt;7.0</td>
</tr>
<tr>
<td>Sows mated within 7 days of weaning, %</td>
<td>90.0</td>
<td>&lt;85.0</td>
</tr>
<tr>
<td>Empty days/sow/year</td>
<td>12.0</td>
<td>&gt;20.0</td>
</tr>
<tr>
<td>Total piglets born/litter</td>
<td>12.0</td>
<td>&lt;11.0</td>
</tr>
<tr>
<td>Piglets born alive</td>
<td>11.3</td>
<td>&lt;10.5</td>
</tr>
<tr>
<td>Mean piglet birth weight, kg</td>
<td>1.4</td>
<td>&lt;1.1</td>
</tr>
<tr>
<td>Pre-weaning mortality, %</td>
<td>&lt;10.0</td>
<td>&gt;13.0</td>
</tr>
<tr>
<td>Piglets weaned/litter</td>
<td>10.2</td>
<td>&lt;9.5</td>
</tr>
<tr>
<td>Piglets weaned/sow/year</td>
<td>24.5</td>
<td>&lt;21.0</td>
</tr>
<tr>
<td>Pigs sold/sow/year</td>
<td>23.0</td>
<td>&lt;19.0</td>
</tr>
<tr>
<td>Piglet weaning weight, kg * (day 23)</td>
<td>7.0</td>
<td>&lt;6.0</td>
</tr>
<tr>
<td>Litter weaning weight, kg * (day 23)</td>
<td>70.0</td>
<td>&lt;60.0</td>
</tr>
<tr>
<td>Feed/sow/year, tonne</td>
<td>1.20</td>
<td>&lt;1.00, &gt;1.5</td>
</tr>
<tr>
<td>Sow feed/piglet weaned, kg</td>
<td>5.0</td>
<td>&gt;5.5</td>
</tr>
</tbody>
</table>

* These are dependent on the age at weaning

A major factor limiting the number of piglets produced per sow per year is the number of litters per sow per year, which is a reflection of the number of empty or non-productive days. These are expensive in terms of lost productivity; and some idea of the economic loss for different sow herd sizes is provided in Table 4. Thus, another important objective of the Premier Pig Program™ is to provide procedures that allow calculation of the cost of lost productivity, such as that associated with the number of empty or non-productive days.

The nutritional and management needs of the boar are different from those of the sow and these are discussed. Strategies need to be developed to optimise semen quality, since this influences fertilisation rate and hence, potential litter size. A summary of the major factors that influence sow reproductive performance are given in Table 5.

The weaner piglet

Weaning is a major challenge to the young piglet and represents a critical period in its life. In nature, weaning is a gradual process that is completed at some 10-12 weeks of age. In commercial practice, weaning normally takes place at between 14 and 28 days of age, or sometimes later, and is an abrupt process that has considerable consequences for the piglet, involving substantial changes in its metabolic, physiological, endocrine and immune processes and greatly affecting its subsequent growth and health status.

Table 4. Example calculation of the economic loss associated with empty or non-productive days.

<table>
<thead>
<tr>
<th>No. of empty days/parity*</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litters/sow/year</td>
<td>2.45</td>
<td>2.30</td>
<td>2.16</td>
<td>2.04</td>
<td>1.93</td>
</tr>
<tr>
<td>Piglets reared/sow/year¹</td>
<td>24.5</td>
<td>23.0</td>
<td>21.6</td>
<td>20.4</td>
<td>19.3</td>
</tr>
<tr>
<td>Reduction in piglets weaned</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Value of piglets, €</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Empty days/sow/year</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penalty/empty day, €</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potential losses by sow herd size:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>250 sows</td>
<td>11,250</td>
<td>39,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 sows</td>
<td>22,500</td>
<td>78,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,000 sows</td>
<td>45,000</td>
<td>156,000</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Assumes a parity of 149 days: 114 days gestation + 28 days lactation + 7 days wean-mating
¹ Assumes 10 piglets reared/litter
² Value of piglet = €30
*Values may change depending on country
Table 5. Suggested feeding and management strategies to optimize sow productivity.

1. Select gilts as early as possible; important for acclimatisation
2. Feed special gilt rearing diet: flush-feed before mating (high ovulation rate)
3. 1st mating at: 220 - 230 days of age
   130 - 140 kg body weight
   16 - 20 mm P2 (condition score: 3.0)
4. Reduce feed intake in gilts for 3 weeks post-mating (high embryo survival)
5. Then feed to body condition target condition score at farrowing 3.5 (scale 1-5)
6. If temperature <20°C, increase feed allowance by 4% per 1°C below 20°C
7. Increase feed intake over last 4 weeks of gestation to optimise birthweight and mammary development
8. Reduce feed 1-2 days before farrowing to facilitate farrowing process
9. After farrowing: gradually increase daily intake over first week; then feed to appetite
10. Good appetite during lactation is critical for high piglet weaning weight
11. Mean requirements during lactation 100 MJ DE (23 Mcal ME) and 60 g lysine/day
12. Minimise loss of body weight and condition. Target condition score at weaning 2.5 (scale 1-5)
13. Piglet weaning weight: 7 kg at 23 days. Each 1 kg litter growth rate requires 4 l of milk
14. Feed separate gestation and lactation diets:
   - Gestation: 13.0 MJ DE (3.0 Mcal ME) and 6 g lysine/kg
   - Lactation: 13.5 - 14.5 MJ DE (3.1 - 3.3 Mcal ME) and 8 - 11 g lysine/kg
15. Lactating sows need 30-50 l water/day; nipple flow rate >2.0 l/min
16. Reduce demand on sow provide supplementary feeding to piglets, cross-foster or split-wean
17. Reduce empty or non-productive days: each day costs ~ 2.0 €
18. Organic minerals boost reproductive performance. Role of: Se, Cr, Fe and SowPak™
19. Ensure good sow health and welfare. Role of Bio-Mos®, also good hygiene
20. Don’t forget the boar! Semen quality, high fertilisation rate, high litter size

The Premier Pig Program™ therefore discusses:

- The metabolic, endocrine and physiological changes that occur at weaning and how these may be manipulated for the benefit of the piglet
- Growth potential and target levels of performance
- How the nutritional needs can best be met and feed and water management
- Ways to enhance appetite post weaning
- Environmental and housing requirements
- How to ensure a high immune and health status
- The importance of good management, husbandry and healthcare practices.

If the effects at weaning are minimised, then it is possible to produce a 30 kg piglet at 10 weeks of age. The target levels of performance at the different stages post-weaning needed to achieve this are suggested in Table 6.

Table 6. Suggested target levels of performance for the piglet post-weaning.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Body weight (kg)</th>
<th>Feed intake (g/day)</th>
<th>Growth rate (g/day)</th>
<th>Feed : gain (g/g)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-35</td>
<td>7 - 10.5</td>
<td>250</td>
<td>250</td>
<td>1.0</td>
<td>&lt;3</td>
</tr>
<tr>
<td>35-49</td>
<td>10.5 - 17</td>
<td>575</td>
<td>450</td>
<td>1.3</td>
<td>&lt;1.5</td>
</tr>
<tr>
<td>49-70</td>
<td>17 - 30</td>
<td>900</td>
<td>600</td>
<td>1.5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Overall</td>
<td>7 - 30</td>
<td>620</td>
<td>460</td>
<td>1.35</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

The importance of weaning weight and performance in the post-weaning period, and their effects on subsequent growth rate and performance, are recognised and ways on how to achieve this are suggested in Table 7.

The grower/finisher pig

The modern pig has a high potential for growth and protein or lean gain. Indeed, growth rates in excess of 1.2 kg/day and protein gains greater than 200 g/day have been achieved in the grow-finish period under ideal conditions. Although these rates are seldom achieved in practice, the potential for growth is higher than that currently achieved on many farms and target levels are suggested in Table 8. If these levels of growth are not achieved, then extra feed is
Table 7. Suggestions for achieving good piglet performance post-weaning.

1. Maximise weaning weight: target 7 kg at 23 days (appropriate sow feeding)
2. Provide supplementary feeding when sow milk supply is inadequate or litter size is >10
3. Pen according to weaning weight and size
4. Special care for smallest piglets (or delay weaning)
5. High feed intake post weaning is critical
6. Growth rate must be at least 200 g/day to maintain body fat reserves
7. Feeding high quality diets of correct nutrient specification is essential
8. Phase diets to piglets needs and digestive competence
9. Feed a little and often just after weaning, with sufficient trough space (50 mm/pig)
10. Feeder hygiene is critical: clean at least twice per day immediately post-weaning
11. Wet (gruel) feeding for first few days is beneficial
12. Adequate number of drinkers (10 piglets per bowl): water flow rate 1.0 l/minute
13. Temperature: initially 28°C; then reduce by 2°C each week until 20°C. If housed in shelters, provide ample straw bedding material and creep area
14. Maintain good air quality: do not reduce ventilation to maintain temperature
15. Avoid draughts and wet floors
16. Do not overstock: each piglet needs 0.20 m² in conventional housing, or 50% more if in alternative bedding systems
17. Lighting: leaving lights on for the first few days may help
18. Promote good health and immunity: treat sick animals promptly
19. Good hygiene, cleanliness and disinfection between batches is essential
20. There must be a high degree of stockmanship

Table 8. Suggested targets for the grower-finisher pig under good commercial conditions.*

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Body weight (kg)</th>
<th>Feed intake (kg/d)</th>
<th>Growth rate (kg/d)</th>
<th>Feed : gain (kg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56-84</td>
<td>20-40</td>
<td>1.4</td>
<td>0.70</td>
<td>2.0</td>
</tr>
<tr>
<td>84-108</td>
<td>40-60</td>
<td>1.9</td>
<td>0.83</td>
<td>2.3</td>
</tr>
<tr>
<td>108-129</td>
<td>60-80</td>
<td>2.4</td>
<td>0.95</td>
<td>2.5</td>
</tr>
<tr>
<td>129-149</td>
<td>80-100</td>
<td>2.8</td>
<td>1.00</td>
<td>2.8</td>
</tr>
<tr>
<td>149-170</td>
<td>100-120</td>
<td>3.0</td>
<td>0.93</td>
<td>3.2</td>
</tr>
<tr>
<td>Overall</td>
<td>20-120</td>
<td>2.2</td>
<td>0.87</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*Assumes a good rate of protein or lean gain, with a P2 value of 10-12 at 100 kg body weight and 12-14 mm P2 at 120 kg body weight.

Meat quality

Factors influencing both carcass and meat quality are discussed, including the components of meat quality. The nutritional and managemental procedures that can be employed to ensure a more consistent product of good meat eating quality with desirable flavour and good keeping and hygiene quality are highlighted.

Environmental issues

Interest in the environment has intensified in recent years and there is much discussion on how to reduce environmental pollution from whatever source. With regard to pig production, the major concerns are the required and the time taken to reach slaughter weight is increased. This increases the cost of production as shown in Table 9. Ways to achieve good performance are presented in the Premier Pig Program™. A summary of the major factors that influence the performance of the grower-finisher pig, and which are discussed in the Premier Pig Program™, is presented in Table 10.

Table 9. The predicted cost of reduced growth rate (20-100 kg body weight).

<table>
<thead>
<tr>
<th>Feed intake (kg/d)</th>
<th>Growth rate (g/d)</th>
<th>Days</th>
<th>Feed (kg)</th>
<th>Extra feed*</th>
<th>Value*</th>
<th>Extra overhead</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00</td>
<td>700</td>
<td>114.3</td>
<td>228.6</td>
<td>44.6</td>
<td>8.9</td>
<td>3.4</td>
<td>12.3</td>
</tr>
<tr>
<td>2.05</td>
<td>750</td>
<td>106.7</td>
<td>218.7</td>
<td>34.7</td>
<td>6.9</td>
<td>2.7</td>
<td>9.6</td>
</tr>
<tr>
<td>2.10</td>
<td>800</td>
<td>100.0</td>
<td>210.0</td>
<td>26.0</td>
<td>5.2</td>
<td>2.0</td>
<td>7.2</td>
</tr>
<tr>
<td>2.15</td>
<td>850</td>
<td>94.1</td>
<td>202.3</td>
<td>18.3</td>
<td>3.7</td>
<td>1.4</td>
<td>5.1</td>
</tr>
<tr>
<td>2.20</td>
<td>900</td>
<td>88.9</td>
<td>195.6</td>
<td>11.6</td>
<td>2.3</td>
<td>0.9</td>
<td>3.2</td>
</tr>
<tr>
<td>2.25</td>
<td>950</td>
<td>84.2</td>
<td>189.5</td>
<td>5.5</td>
<td>1.1</td>
<td>0.4</td>
<td>1.5</td>
</tr>
<tr>
<td>2.30</td>
<td>1000</td>
<td>80.0</td>
<td>184.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Comparisons are made with a pig growing at 1000 g/day and having a mean feed intake of 2.3 kg/day
*Cost of feed € 200/ton. Each extra day costed at € 0.1/day
*Values may change depending on country
excretion of excessive nitrogen and phosphorus and the emission of noxious gases, such as NH₃, CO, CO₂ and H₂S. Ways to minimise these pollutants are discussed.

Application of the Premier Pig Program™

The program was launched in New Zealand and Australia during 2002 and 2003 as a series of three workshops dealing with the breeder, weaner and grower/finisher pig. Approximately 1,000 people attended these workshops, representing 90% of the Australian and New Zealand pig population. The positive response from and uptake by the industry was such that the program has now been launched in Asia and Europe with considerable success.

The Premier Pig Program™ has been designed to continuously evolve. As new research results and technical data become available, updates to the manual will be prepared and distributed. In this way,
the manual will be kept up-to-date and relevant. The overall objective is to provide practical information in order to ensure long-term efficiency and profitability of pig production.

Acknowledgement

It is a pleasure to thank Dr. Bruce Mullan, Department of Agriculture, Western Australia, Mr. Tony Edwards, A.C.E. Livestock Consulting Pty. Australia and Dr. Kate Jacques, Alltech Inc. Lexington, KY, USA, for their contributions to the development and application of this program.
Successful feed companies in the future

JIM HEDGES

Hubbard Feeds Inc., Mankato, Minnesota, USA

Like everything else, the feed industry has changed. I cannot think of a lot that has not changed. Most of our people use notebook computers. The original ones were called portable computers and we joked that they should have been called ‘luggable’. The early models were about the size of a small to medium-sized suitcase and were certainly not light weight. Wal-Mart has taken the place of Woolworth and K-Mart. They offer it all with one stop, from food to motor oil to clothes. They evolved with the times; and sell good products at very reasonable prices. Wal-Mart has excellent marketing and they recognize trends and change with the times and customer needs and wants.

The feed company that succeeds must be as innovative and market-driven as the computer industry and a Wal-Mart Superstore. I have always tried to pattern our work after the electronic industry. There are similarities, but the electronics companies have some advantages. A VCR 20 years ago retailed for approximately $780, while today the price is about $80 and quality is vastly improved. Research in food animal nutrition has always been driven toward maintaining performance while lowering costs. Unlike electronic companies, we cannot control the commodity market and therefore have less control over input costs, but we have made great strides. Two examples of ways we have gained control over feed ingredients costs is with the enzyme phytase and through the use of synthetic amino acids.

The January 1903 Feed Management magazine article on the Top Feed Manufacturers is quite enlightening (Table 1). In 2001 the magazine expanded the survey to include leading integrated poultry and livestock producers. In the 2002 edition of the article, only four of the top 10 feed manufacturers are actually feed companies. The top manufacturer is a conglomeration of what used to be three quite large individual feed companies, namely Land O’ Lakes, Farmland Feed and Purina Mills. How did this come to pass? In part it is due to traditional feed companies failing to supply what the customer wanted. My theory is that if the major feed companies had produced and marketed premixes when the market demanded the product, it would have been very difficult for the strictly premix companies to have developed and grown. The big traditional feed companies had the sales force, technical expertise and dealer network to fill the market need, but were afraid they would have several mills sitting idle. The moral of the story is you cannot make people buy what you want to sell. At Wayne Feed when we finally introduced premix in the early 1980s, we actually increased market share by not only gaining customers with the premix, but by selling more concentrate as well. Without a premix, it was easy for the producer (potential customer) to dismiss a salesman. Once we had a premix to offer, many producers, when offered the option of either premix or concentrate from the same company changed back to a concentrate-feeding program since many of the producers in the early 1980s really were not big enough to conventionally use premix and soybean meal.

One very important point concerning the list of top feed manufacturers is that the ranking is based on production capacity, not actual production. In this ranking, the North American Animal Nutrition Companies (most of you know them as Akey) is ranked number 52. I dare say there are very few feed companies that feed more animals than this one. This suggests that bricks and mortar are not necessarily needed to sell and service a significant portion of the livestock industry.

The 2003-04 Feedstuffs Reference Issue and Buyers
Successful feed companies in the future

Guide lists the top 10 feed manufacturers and is more pertinent for this paper (Table 2). Again, the ranking is based on manufacturing capacity, not sales. Ridley Inc. is ranked No. 9, but if this ranking was based on animals fed, there would be very few feed companies ahead of Ridley.

Table 2. Top US feed companies, based on manufacturing capacity, mid-2003.

<table>
<thead>
<tr>
<th>Company name &amp; headquarters locations</th>
<th>Annual manufacturing capacity (thousand tons/yr)</th>
<th>States served</th>
<th>Complete (%)</th>
<th>Pelleted (%)</th>
<th>Bulk (%)</th>
<th>Dealer-sold (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Land O’Lakes Farmland Feed/Purina Mills Arden Hills, MN</td>
<td>12,881</td>
<td>48</td>
<td>64</td>
<td>54</td>
<td>71</td>
<td>52</td>
</tr>
<tr>
<td>Tyson Foods Rogers, AR</td>
<td>12,000</td>
<td>40</td>
<td>100</td>
<td>78</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Cargill, Inc. Minneapolis, MN</td>
<td>9,500</td>
<td>49</td>
<td>55</td>
<td>65</td>
<td>65</td>
<td>60</td>
</tr>
<tr>
<td>Smithfield Foods, Inc., Smithfield, VA</td>
<td>4,500</td>
<td>100</td>
<td>85</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ADM Alliance Nutrition, Inc. Quincy, IL</td>
<td>3,200</td>
<td>42</td>
<td>50</td>
<td>57</td>
<td>67</td>
<td>59</td>
</tr>
<tr>
<td>Pilgrim’s Pride Corp. Pittsburg, TX</td>
<td>3,190</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Perdue Farms, Inc. Salisbury, MD</td>
<td>3,016</td>
<td>12</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gold Kist, Inc. Atlanta, GA</td>
<td>3,000</td>
<td>5</td>
<td>100</td>
<td>91</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>J.D.Heiskell &amp; Co. Tulare, CA</td>
<td>2,800</td>
<td>6</td>
<td>65</td>
<td>5</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>ConAgra Poultry Co. Duluth, GA</td>
<td>2,180</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*2*Includes Canadian feed tonnage

Premix companies hardly even make it into these rankings, but again if this were based on animals fed, several of these companies would be near the top. These rankings are interesting, but capacity has nothing to do with sales success. Actually in today’s market it could be argued that milling capacity could be a negative for a feed company. It is extremely difficult to be cost competitive and profitable while maintaining a lot of mills operating at 25 to 50% capacity.

As a normal rule, feed manufacturers cannot make feed as cheaply as a large livestock producer. A livestock producer with a modern mill can make significantly more tons of feed per hour than a feed manufacturer. The livestock producer may have only 10-20 base formulas of meal feed made in 3-5 ton batches in bulk while the commercial feed company mill may have 200 formulas. Also, most feed companies are multi-species, which increases the complexity of production. Being multi-species not only affects the number of formulas, but can significantly affect sequencing and even the raw material inventory. For several years now Ridley has had a policy to not inventory any ruminant meat and bone meal or even plasma of ruminant origin. With these issues on ingredients, products, labor, taxes etc., the large commercial feed mill cannot compete on a price per ton basis with single species or a simple meal-producing mill.

Given these constraints, how can feed companies
grow and be successful? Most feed companies are trying to grow by acquisition. Definitely this can accomplish the goal, but is not without risk. When a company that has been a competitor for years is purchased, differing company cultures usually clash. Hubbard has gained significant new business from the gridlock that can occur with these acquisition patterns in the marketplace. Slow decision-making and poor service while two companies decide who is in charge has enabled us to obtain huge volumes of business. One customer asked for a loan and he never received a response. He told me he could understand a ‘no’, but no response at all after being a customer for 7-8 years drove him away. In another instance we gained 2000 tons of new business per month when one company bought another. Management, in many cases, does not seem to realize that change cannot be forced on people. If a customer, either a feed dealer or direct account, is forced to change their way of doing business, why not change completely to another company? These customers (dealers) perceived their original brand being de-emphasized and felt steamrolled into the new brand. On top of this were poor service and poor communication. The result was a change to a company that wanted their business and supported them. You can buy a company, its mills, trucks and offices, but its dealers and direct customers do not automatically follow. Companies spend a tremendous amount of time and money developing a company culture and image. It is neither easier nor a good idea to discard it without recognizing its value in the marketplace. When the old Hubbard bought companies they made the transitions relatively successfully, keeping the original brands until time and market changes deemed it good management practice to bring the brands into the parent company. It is critical to understand that we can buy an employee’s time; or a business; but simply ‘buying’ is not effective. Only voluntary actions, those which cannot be bought, are ultimately effective. Trust, for example, must be earned. You earn trust by consistently doing what is right for the long term, not just for the next quarter’s results. Customers need time to adjust to the new company, because they make decisions for their own reasons, not ours. You grow the business by infusing capital and technically improving the products. Making more than one brand of feed at the same plant admittedly causes some difficulties, but the dealers will be somewhat more tolerant as long as service is acceptable and feed quality is good. Brand names are important to dealers; and if you are to change the brand name, the dealer needs time to accept this change. The change can be made considerably less painful with effective training and product positioning. When Ridley bought the Wayne Feed brand we did not force the Wayne dealers to convert to the Hubbard brand immediately. We updated the products and said that as long as they sold the existing brands in good volume to keep on selling them. As dealers undergo more and more training sessions and have updated products to offer, they will change to new products without excess trauma. They must be confident the changes benefit the customer, not just the parent company.

While product brands are important to dealers and small to medium size producers, they are not the main issues with large producers. For all your customers, the products must meet a need either through technology advances or by providing something the customer cannot make himself. Unless your goal is toll milling, complete grow-finish or sow diets are not a growth option for most of today’s feed companies. For this segment of the market, it is best to supply some type of premix and possibly ingredients. To prevent this from being a complete bid situation, you need excellent technical support and formulation help. The diet density and feed budgets are a part of what the successful feed company must also supply. For the megaproducers, products are somewhat different for each customer depending on the customer’s feed milling capabilities. We have customers who require a 10 lb/ton vitamin/trace mineral premix sent to some of their toll mills and a 60 lb/ton base mix for other mills. We have other customers with sophisticated mills that we supply with a 2 lb/ton vitamin/trace mineral premix. If a livestock producer has a $10 million feed mill, he will not be buying a 60 lb/ton base mix. There is room for both segments; you do what the customer needs and respect his milling capabilities.

One customer need that a feed company can meet is in supplying feed milling technology. Most producers need help in this area as well as in monitoring ingredient quality. They also need help in monitoring finished feed quality. There are a lot of large dealers or co-op mills that have tremendous grain handling capability and milling capacity, but do not have the nutrition technology that progressive pork producers need. This represents another market segment for the traditional feed company, but it differs in that these are not the same products the company supplied years ago. In these situations we supply the vitamin/trace mineral premix or base mix and do the finished diet formulation while the toll millers do the grinding, mixing and delivery.

The starter feed area is definitely an area where there
Successful feed companies in the future

is opportunity for differentiation from the competition. The early-stage complex diets are difficult to manufacture. In addition, most swine producers do not have storage capacity for all the ingredients needed for these feeds, and certainly do not possess the technology to make them.

Research

From a technical perspective, this is a key to a successful future. In the past, feeds and feed ingredients could be sold based on testimonials, but this approach no longer suffices. Statistically valid trials must be conducted in facilities and with genetics similar to those of your customers. We made mistakes years ago with facilities that were ‘ideal’ for research, but the results did not apply to the field. The data in Tables 3 and 4 show this very well. Notice the huge difference in feed intake between the two groups. Pigs housed in the commercial unit stocked at 45 pigs/pen consumed 5.1 lbs/day compared to 6.2 lbs/day for the research facility pigs stocked at 10 pigs per pen. Feed conversion is not greatly different, but the data for the research-stocked pigs indicated these pigs needed only 0.49% available lysine (the lowest level fed) and the commercial-stocked pigs need 0.65% available lysine! Our research facilities once had a limited number of pigs per pen. The performance of the pigs in these facilities was great, yet the salesmen and dealers complained the commercial hogs stalled out and did poorly in many cases. The diets were correct, but only under the circumstances in which they were fed.

Table 3. Research pens, 10 pigs/pen.

| Avail. lys, % | 0.49 | 0.65 | 0.77 | 0.88 |
| Tot. lys, %  | 0.60 | 0.77 | 0.90 | 1.04 |
| St wt, lbs   | 136.7| 140.6| 146.1| 141.7|
| End wt, lbs  | 236.8| 241.6| 247.1| 240.7|
| ADG, lbs     | 2.32 | 2.35 | 2.34 | 2.30 |
| ADFI, lbs    | 6.26 | 6.41 | 6.28 | 6.10 |
| F/G          | 2.68 | 2.73 | 2.70 | 2.66 |
| P2           | 0.77 | 0.76 | 0.78 | 0.77 |

Campbell (1995)

Table 4. Commercial pens, 45 pigs/pen.

| Avail. lys, % | 0.49 | 0.65 | 0.77 | 0.88 |
| St wt, lbs    | 119.2| 119.7| 120.6| 119.2|
| End wt, lbs   | 191.8| 197.5| 196.6| 195.5|
| ADG, lbs      | 1.81 | 1.94 | 1.90 | 1.91 |
| ADFI, lbs     | 5.14 | 5.11 | 5.13 | 5.13 |
| F/G           | 2.83 | 2.63 | 2.70 | 2.69 |
| P2            | 0.53 | 0.49 | 0.50 | 0.50 |

Campbell (1995)

Table 5 illustrates the importance of feed intake adjustments needed to ensure that pigs receive 20 g of lysine per day. This is an old concept, but many people do not understand it to this day. The data in Figure 1 show how adjusting the diet can alleviate part of the performance depression that can occur in restricted feed intake situations. The optimum lysine level for these finishing hogs when fed \textit{ad libitum} is 0.61%. When the hogs are restricted to 85% of \textit{ad libitum} intake, the optimum lysine level is 0.85% of the diet. A reduction to 85% of \textit{ad libitum} is not an academic exercise. Our large customers running mills 20 hrs a day do not have to run that hard in July and August, when feed intake falls due to heat stress. Not all of the reduction in performance due to heat stress can be overcome, but it can be alleviated to some extent by diet manipulation.

Table 5. Effect of feed intake on daily lysine intake in finishing hogs.

<table>
<thead>
<tr>
<th>Daily feed intake (lb)</th>
<th>Lysine/day (g)</th>
<th>Lysine needed to supply 20 g/day crude protein (%)</th>
<th>Approximate crude protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0</td>
<td>20.6</td>
<td>0.65</td>
<td>14.0</td>
</tr>
<tr>
<td>6.5</td>
<td>19.2</td>
<td>0.70</td>
<td>14.5</td>
</tr>
<tr>
<td>6.0</td>
<td>17.7</td>
<td>0.75</td>
<td>15.5</td>
</tr>
<tr>
<td>5.5</td>
<td>16.2</td>
<td>0.83</td>
<td>16.5</td>
</tr>
<tr>
<td>5.0</td>
<td>14.8</td>
<td>0.91</td>
<td>17.5</td>
</tr>
</tbody>
</table>

Hedges (1994), unpublished

Table 6 illustrates the reference data in Table 6 are accurate under the conditions in which the pigs were fed, but the diet densities recommended would probably not give optimum performance in a large commercial unit. The swine producer does not have the luxury of removing the pigs that are too big or too little; the producers feed all the pigs.

Early in my career the salesmen accused me of doing ‘Ivory Tower’ research. Actually, they were correct. There has always been a bit of disconnect between university recommendations and the feed industry. There is no need to have a big debate over this. I am not trying to criticize the excellent scientists that did this work, I just do not think you should rely on university tables for all nutrient recommendations. The point is, if you are to have industry-leading diets, you must develop them yourself. From my perspective, the role of the university is to do the basic work. I have formulated swine diets for 25 years using digestible amino acid values and I did not generate any of the values I used. All the digestible amino acid data was generated by university research; which we applied and made practical. We use the enzyme
Table 6. Suggested dietary amino acid and protein allowances for swine fed corn-soybean meal diets at various bodyweights.

<table>
<thead>
<tr>
<th>Item</th>
<th>20-45</th>
<th>45-80</th>
<th>80-120</th>
<th>120-170</th>
<th>170-220</th>
<th>220-280</th>
<th>120-170</th>
<th>170-220</th>
<th>220-280</th>
<th>Gestation</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barrows and gilts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>1.20</td>
<td>1.00</td>
<td>0.90</td>
<td>0.75</td>
<td>0.68</td>
<td>0.58</td>
<td>0.84</td>
<td>0.73</td>
<td>0.62</td>
<td>0.55</td>
<td>0.92</td>
</tr>
<tr>
<td>Met+Cys(^6)</td>
<td>0.72</td>
<td>0.62</td>
<td>0.56</td>
<td>0.48</td>
<td>0.43</td>
<td>0.37</td>
<td>0.53</td>
<td>0.47</td>
<td>0.39</td>
<td>0.30</td>
<td>0.44</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.21</td>
<td>0.18</td>
<td>0.16</td>
<td>0.15</td>
<td>0.13</td>
<td>0.11</td>
<td>0.16</td>
<td>0.14</td>
<td>0.11</td>
<td>0.11</td>
<td>0.16</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.78</td>
<td>0.67</td>
<td>0.60</td>
<td>0.52</td>
<td>0.48</td>
<td>0.40</td>
<td>0.58</td>
<td>0.51</td>
<td>0.43</td>
<td>0.45</td>
<td>0.58</td>
</tr>
<tr>
<td>Arginine(^6)</td>
<td>0.50</td>
<td>0.36</td>
<td>0.33</td>
<td>0.14</td>
<td>0.13</td>
<td>0.10</td>
<td>0.15</td>
<td>0.14</td>
<td>0.11</td>
<td>0.00</td>
<td>0.50</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.39</td>
<td>0.32</td>
<td>0.29</td>
<td>0.24</td>
<td>0.21</td>
<td>0.19</td>
<td>0.27</td>
<td>0.23</td>
<td>0.20</td>
<td>0.17</td>
<td>0.35</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.72</td>
<td>0.60</td>
<td>0.54</td>
<td>0.45</td>
<td>0.40</td>
<td>0.35</td>
<td>0.50</td>
<td>0.44</td>
<td>0.37</td>
<td>0.31</td>
<td>0.50</td>
</tr>
<tr>
<td>Valine</td>
<td>0.81</td>
<td>0.68</td>
<td>0.61</td>
<td>0.51</td>
<td>0.47</td>
<td>0.39</td>
<td>0.57</td>
<td>0.50</td>
<td>0.42</td>
<td>0.36</td>
<td>0.76</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.20</td>
<td>1.00</td>
<td>0.90</td>
<td>0.75</td>
<td>0.68</td>
<td>0.58</td>
<td>0.84</td>
<td>0.73</td>
<td>0.62</td>
<td>0.45</td>
<td>0.96</td>
</tr>
<tr>
<td>Phe+ Tyr(^7)</td>
<td>1.14</td>
<td>0.95</td>
<td>0.86</td>
<td>0.71</td>
<td>0.65</td>
<td>0.55</td>
<td>0.80</td>
<td>0.70</td>
<td>0.59</td>
<td>0.50</td>
<td>1.00</td>
</tr>
<tr>
<td>Protein(^8)</td>
<td>20</td>
<td>18</td>
<td>16</td>
<td>14</td>
<td>13</td>
<td>12</td>
<td>16</td>
<td>14</td>
<td>13</td>
<td>12</td>
<td>17</td>
</tr>
</tbody>
</table>

1Bodyweights are listed in pounds. The suggested protein and amino acid allowances assume that corn-soybean meal diets are fed, and that the diets contain 1,560 and 1,500 kcal/lb DE and ME, respectively. Ideal ratios (relative to lysine) were used in calculating amino acid requirements for market pigs considered to be of high-lean genetics.
2Developing boars should receive 0.90 and 0.75% lysine during the grower and finisher phases, respectively. Specific requirements for the mature boar have not been established. A diet that is adequate for the gestating gilt should be adequate for the mature boar.
3A minimum of 28 days lactation is assumed.
4These allowances were determined with fortified corn-soybean meal diets. Substitution of other grains for corn or other protein sources for soybean meal should be made on a digestible amino acid basis.
5Cystine can supply up to 50% of the requirement for sulfur amino acids. Thus, the values given here represent a Met + Cys allowance.
6Dietary arginine is not essential for gestation.
7Tyrosine can satisfy 50% of the need for total aromatic amino acids (phenylalanine + tyrosine).
8These levels of crude protein in a corn-soybean meal diet will generally meet the amino acid requirements, although some supplemental lysine may be needed.

Source: Baker et al., 2003.

Figure 1. Ad libitum vs restricted feeding: effect on required dietary lysine concentrations (adapted from Coma et al., 1995).
Successful feed companies in the future

Phytase, the efficacy data on which was generated by the universities in their facilities with graduate students available. The feed industry nutritionist should be able to take this basic work and figure out how it can be applied in the commercial world. The vast majority of our research is conducted in barns with 1000 or more pigs, in industry standard pen sizes. This allows the pigs to experience the same space, social and ventilation factors they would encounter in normal commercial units. These facilities are all commercial facilities with modifications for weighing both feed and pigs accurately.

We have a 1000-head capacity research nursery that is a contract barn for a large producer. This barn has pen scales, six feed tanks instead of one and a Mosdal feed cart with built in scales. The pigs are weaned at approximately 20 days of age and transported 12 to 15 hrs to this nursery. When the pigs arrive they are weighed and allotted to treatments, without an adjustment period. The diets are formulated to meet the nutritional needs of pigs housed at 22-25 pigs per pen. The data in Table 7 show the performance difference that can be obtained with different starter feed formulations. The university diet is much simpler and represents the optimum formulation for a university nursery with 10 pigs/pen. The other feed companies’ diets were probably also developed in small pen nurseries. These trials are more for in-house benchmarking of performance versus the competition than for selling. They are not great sales tools; producers’ interest might be aroused, but normally they do their own tests. In another example Table 8 shows what complex diets do for uniformity of gain. Very healthy pigs with minimal stress can grow quite well with less complex diets, except this is not the normal situation in the commercial world.

The grow-finish research barns are actually commercial 1000 or 1200-head barns modified with scales for weighing the hogs and equipment to measure feed intake. Depending on the facility, the hogs are housed 20 to 32 pigs/pen with normally 7.5 ft²/hog. All dietary treatments are replicated 6 to 8 times. A couple of these barns have dual water lines for studying water treatments as well. In total, Ridley has two 1000-head conventional nurseries for research and three wean-to-finish barns of 1000 head

| Table 7. Starter diet comparison report sheet from a contract nursery. |
|-------------------------|-----------------|--------|--------|--------|--------|----------|
|                         | HFI            | New HFI | Brand A | Brand B | Brand C | University |
| Start wt                | 11.01          | 11.01   | 11.06   | 11.08   | 11.03   | 11.03     |
| Day 4                   |                |         |         |         |         |           |
| ADG, lbs                | 0.71           | 0.71    | 0.7     | 0.55    | 0.63    | 0.61      |
| F/G                     | 0.62           | 0.62    | 0.63    | 0.62    | 0.64    | 0.68      |
| ADFI, lbs               | 0.43           | 0.43    | 0.43    | 0.33    | 0.39    | 0.39      |
| Day 4-11                |                |         |         |         |         |           |
| ADG lbs                 | 0.63           | 0.63    | 0.37    | 0.55    | 0.62    | 0.49      |
| F/G                     | 1.21           | 1.21    | 1.54    | 1.19    | 1.20    | 1.32      |
| ADFI lbs                | 0.76           | 0.76    | 0.55    | 0.65    | 0.74    | 0.64      |
| Day 11 wt., lbs         | 18.29          | 18.29   | 16.45   | 17.16   | 17.87   | 16.91     |
| Day 11-25               |                |         |         |         |         |           |
| ADG lbs                 | 0.98           | 1.08    | 1.02    | 1.08    | 0.96    | 0.82      |
| F/G                     | 1.55           | 1.37    | 1.39    | 1.37    | 1.48    | 1.54      |
| ADFI lbs                | 1.52           | 1.48    | 1.41    | 1.47    | 1.42    | 1.26      |
| Day 25 wt., lbs         | 32.06          | 33.35   | 30.67   | 32.26   | 31.34   | 28.42     |
| Day 0-39                |                |         |         |         |         |           |
| ADG lbs                 | 1.06           | 1.09    | 1.02    | 1.03    | 1.02    | 0.94      |
| F/G                     | 1.52           | 1.48    | 1.44    | 1.37    | 1.5     | 1.48      |
| ADFI lbs                | 1.61           | 1.61    | 1.47    | 1.41    | 1.52    | 1.39      |
| Day 39 wt., lbs         | 52.16          | 53.49   | 50.88   | 51.08   | 50.67   | 47.7      |
| Feed budget             |                |         |         |         |         |           |
| Nursery 1               | 1              | 1       | 0.5     | 1       | 1       | 1         |
| Nursery 2               | 3              | 3       | 1.5     | 3       | 3       | 3         |
| Nursery 3               | 12             | 12      | 12      | 12      | 12      | 12        |
| Nursery 4               | 50             | 50      | 50      | 50      | 50      | 50        |
| Nursery 5               | 0              | 0       | 0       | 0       | 0       | 0         |
Table 8. Determining the differences in nursery feeding programs (Location: Contract nursery).

<table>
<thead>
<tr>
<th>Pens</th>
<th>FC1</th>
<th>Univ. Spec.</th>
<th>HFI</th>
<th>FC2</th>
<th>FC3</th>
<th>FC4</th>
<th>FC5</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Total no. of pigs</td>
<td>92</td>
<td>92</td>
<td>92</td>
<td>92</td>
<td>92</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>Starting weight, lbs</td>
<td>11.03</td>
<td>11.03</td>
<td>11.01</td>
<td>11.08</td>
<td>11.08</td>
<td>11.09</td>
<td>11.06</td>
</tr>
<tr>
<td>Pigs dead or &lt;40 lbs at 39 days</td>
<td>9</td>
<td>15</td>
<td>5</td>
<td>10</td>
<td>3</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Rep 1</td>
<td>8.97</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Rep 2</td>
<td>8.80</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Rep 3</td>
<td>8.62</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Rep 4</td>
<td>11.49</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Rep 5</td>
<td>11.49</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Rep 6</td>
<td>11.49</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Rep 7</td>
<td>12.66</td>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rep 8</td>
<td>13.80</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Rep 9</td>
<td>12.17</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

1Number of times ranked 1b
2Number of times ranked 2
3Number of times ranked 3
4Number of times ranked 4
5Number of times ranked 5
6Number of times ranked 6
7Number of times ranked 7
8Number of times ranked 8
9Number of times ranked 9
10Number of times ranked 10
11Number of times ranked 11
12Number of times ranked 12
13Number of times ranked 13
14Number of times ranked 14
15Number of times ranked 15

* FC = Feed company

** number of pigs that died in a given rep and treatment

** weighted average, the number of times a diet was ranked 1,2, etc. in ADG. HFI five times had best gain, three times second best, one time third, and one time fourth; 5x1+3x2+1x4=15; the lower the number the better.

Customer service

Exceptional customer service is essential for a successful company. Most traditional feed companies have always been only one step away from the end user. Selling direct is different. Dealers buffer the company from the end user. Animals eat 24/7. I know from personal experience that plants and order desk personnel do not always understand urgency. In fairness to the plant personnel, some of our plants are nearly at maximum capacity and it can be quite a juggling act to get all the feed out on time. The time frame is shortened without the dealer’s inventory serving as a buffer between you and the end user.

Dealers have a business history and a brand they have promoted, and tend to have a higher tolerance of ‘average’ service than a direct account. In many cases a direct account communicates personally with the order staff and there is no buffer. Your order staff must be trained to deal with direct customers in a knowledgeable, courteous and efficient manner. Ridley has been adjusting to this change, although there is still room for improvement, as at every company. It seems obvious, but orders must be ready when the customer expects them. Pellets must be excellent and not 10% fines. With many big systems, the pellets are handled several times. If the customer is large enough to have his own pellet mill, you had better deliver significantly better pellet quality than he can make. The successful feed company must have excellent production people and truck drivers.

As a technical salesman it is much easier to sell feed from a plant you know will make the feed correctly. It is one thing to design or formulate a feed on paper, but it must be mixed properly. A mistake with a 5 lb/ton premix is a big mistake, because it will be multiplied many, many times. Proper quality assurance is critical; particularly when the customer must perceive that your service is better than the
Successful feed companies in the future

Researchers Keiningham and Vaura (2001) discuss customer satisfaction in terms of delighted customers rather than just merely satisfied customers. A review of this book suggests that anywhere from 60 to 85% of customers who switched firms would have been classified as satisfied by generally accepted market research measurement tools. The successful organization needs to dramatically increase positive customer experience, virtually eliminate the negatives and drive customers to new levels of repeat purchasing, loyalty and sheer delight. I have often said if we could make excellent pellets, deliver them on time, put them in the correct bin every time, we could not make all the feed we could sell. You do not lose customers, you drive them off. When was the last time you complained about great service?

Management

In the almost thirty years I have been in the feed industry I have worked for about 10 feed division managers. An excellent head of a feed division grows the business, a mediocre one does not hurt the business and a bad one destroys the business. The absolute key element needed for a successful business is people. Without excellent people you have a mediocre or failing company.

The mistake I have seen most often is too much management. The really good salesmen are independent thinkers and do not function well in a rigid structure. In today’s business climate with declining margins, the successful company must have mainly Indians and very few Chiefs. If you are not selling or supporting customers, there is no need for you. Anyone that has contact in any way with customers needs to have good people skills. Customers have enough options to easily avoid buying from people they perceive as difficult. Very little in the company is necessary until something is sold. When I hire a PhD for my group I ask them if they can sell. If they cannot sell, I do not hire them. The PhD nutritionist is a technical salesman in today’s business environment. We have accounts where the only person involved is the technical person and the order desk personnel at the plant. This is partly because the traditional sales force does not have the confidence to call on anyone with 20,000 sows or more. A good account manager can be helpful. They can play a key role in customer support, helping with communicating and listening to the customers needs, concerns and correcting problems. The feed industry used to give away a lot of caps and jackets; and while this is not a big deal with large customers, a shooting outing or fishing trip can be a great rapport builder. People buy from people. It is still a people business whether the customer has 100 sows or 100,000 sows.

Management’s role is to be a leader, a point that seems obvious but is often missed. You do not have to like the feed division general manager, but it is important people respect him. The head person needs to receive and respect feedback from the people doing the selling. He also needs to get out and work with enough customers to know the market needs. The big companies that have failed basically ignored the market and tried to keep on doing what had made them successful. The successful company will have focused, energetic and creative people who can sell without two or three layers of people to tell them what to do. Feed companies used to have huge bureaucracies, but they can no longer afford them. In today’s business environment the big don’t eat the small; the fast eat the slow.

The ideal feed company division head would have the following characteristics:

Be a leader. People are good followers if they have someone they respect to follow. My brother has a saying “you can lead me a long way but you are not going to push me very far”. In my career I have had leaders that tried to motivate by intimidation and it did not work well. To be a good leader you have to listen, support and encourage your people. Get them the tools they need and stay out of the way. Too many general managers overrate themselves; they cannot make the company successful by themselves, without good people they will fail. Poor leaders cannot attract good people.

Listen to your people. I have a fair reputation of being a good product developer, but it has not been hard. The customer will tell you what he needs, you must listen and act. The employees are management’s customers. It is so obvious that I guess it is hard for management to see at times, but all they need to do is listen to the people that are in front of the customers and actually making sales. Having said this, one of the mistakes feed companies make is giving salesmen credit and commission for a huge sale they did not make simply because it is geographically in their territory. Just because the animals are in a
salesman’s territory does not mean he should get credit, but many times sales managers want to protect their salesmen. We cannot afford this anymore. Territory selling is changing to customer selling, and in some cases, team selling.

**Trust your people.** Management must accept that they have employees that want to grow the business just as much as they do; and management should listen and act when these employees request people or equipment to grow the business.

**Be customer friendly.** If in doubt, do what is right for the customer and then work hard to make it right for the company.

**Be able to meet with customers and earn their respect.**

**Be innovative, be able to think outside the box.** It seems most feed companies think the only way to grow is by acquiring another feed company. What is wrong with staffing up and attacking the market with excellent people? Very few feed companies are operating their mills at full capacity. If you cannot grow your existing business, what makes you think you can operate an even larger business?

**Do not be afraid to invest capital to grow the business.** Possibly dedicated feed mills for each species group is the way to go. With a special purpose mill things can be accomplished from a production standpoint that are not possible in a full line plant.

**Conclusions**

I think the feed company that really succeeds in the future will have to quit thinking like this is still 1985. The feed business has changed, but I am not convinced the management at most feed companies realize this - or if they have, they do not know what to do about it. You will need specialists, not generalists. I have never understood the logic of trying to get salesmen to be good at all species. Actually, they are not and they gravitate to the species they are most comfortable with and the one they think they can be the most successful at. The operations are bigger, fewer salesmen may be needed but these individuals need to be of higher quality and be more highly educated.

One thing that has not changed is **you have to call on people to sell.** A lot of salesmen drive by larger operations because they are not confident enough to call on them. This cannot be tolerated if you plan to succeed. Excellent research, product quality (both the formula and the production of it), superior service, and acceptable pricing are all tablestakes. Some company is going to supply the nutrition to the livestock industry; and I think it will be a feed company. I do not think that ingredient companies, amino acid, vitamin or dical suppliers, etc. have the technical expertise or service capability to meet livestock producer needs.

It will be quite interesting 15 years from now to see how the feed industry looks. One area of concern is where are we going to get trained technical people? The old power house universities that produced a lot of good research and trained students in the past have dwindled to a very few. If we can get good people, I think most of the good applied research of the future will be through alliances between the successful feed company and their customers. The large customer and the feed company need to form such alliances. Our customers are not just those we sell to and profit from. They are partners; and need to be thought of as such. It is far too difficult to earn the trust and respect of an account on any other basis. The feed company’s objective is to help make the customer profitable. If the customer fails, the feed company fails also.

Most of this may just seem common sense; but knowing and doing are two different things. The feed business is really quite simple; and maybe that is the problem.

**References**


Successful feed companies in the future


The role of selenium and Sel-Plex® in sow reproduction

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Introduction

The demonstration of selenium (Se) deficiencies in the field was first noted where occurrences of sudden deaths, white muscle degeneration, liver necrosis, mulberry heart, gut-edema, uncontrollable diarrhea, and increased fluid in the pericardial sac were predominant observations in weaned pigs. The condition was not as prevalent with reproducing gilts or sows, for during this period of the swine industry most sows were housed outdoors on concrete pads or in pasture lots. As complete confinement for reproducing animals became more common, increased occurrences of the deficiency in the adult animal began to emerge. Hyposelenosis in the female was generally accompanied by prolonged farrowing times, reduced fertility, increased number of stillbirths, poor milk letdown during the initial days postpartum, higher incidence of MMA (mastitis, metritis, agalactia), lower litter sizes, and greater losses of sows from the herd. Although other factors can also contribute to these clinical signs, current research has confirmed that these observations are related to Se deficiency. Wilkinson et al. (1977) and Mutetikka and Mahan (1993) have confirmed that sows housed on pasture have higher sow serum Se and α-tocopherol concentrations than a similar group of gilts fed in confinement.

Although this dietary level has not been recognized as the dietary requirement for all swine production phases (NRC, 1998), it is the level commonly incorporated into most swine feeds by many feed companies. Over the past two decades, the continued occurrence of vitamin E/Se deficiency in sows and pigs has not been completely abated by adding higher dietary levels of these nutrients, particularly vitamin E, and thus different forms and levels of vitamin E and Se have been more actively pursued.

Selenium deficiency in the sow

Feeding a semi-purified diet deficient in vitamin E and Se resulted in a lower litter size and a low number of sows farrowing (Mahan et al., 1974). Sows fed the diet and their piglets were extremely weak at parturition. None of the sows fed the semi-purified vitamin E/Se deficient diet completed a second parity, but three died during their second pregnancy. Within their reproductive tracts there were both normal and abnormal fetuses, suggesting that tissue atrophy and oxidative damage had occurred. Sows fed the deficient diet also had elevated serum glutamic oxaloacetic acid transaminase (SGOT) enzyme activities by 30 days of gestation, values that increased to yet higher levels as gestation progressed. This enzyme is indicative of cellular damage of sow or fetal tissue. Consequently, these results suggest that diets deficient in Se will deplete sows of body Se and vitamin E reserves, will result in lower litter sizes, reduced sow parturition performance, and increased fetal atrophy and death.

Practical diets fed to reproducing sows generally have not shown evidence of reproductive failure as
readily as when the semi-purified diet was fed. A non Se-fortified corn-soybean meal diet fed to gilts and sows resulted in a lower litter size by parity 2, whereas sows fed the same diet fortified with Se (selenite) at 0.10 ppm had a larger litter size at parity 2 (Mahan et al., 1974). Feeding low Se grains or diets low in vitamin E and Se, particularly grains of a high moisture content, has shown lowered reproductive performance and higher incidence of MMA (Whitehair and Miller, 1985). Most of the published sow data has thus shown a greater effect of supplemental Se in later reproductive parities, with beneficial responses to supplemental Se more in older than in younger sows. This supports the concept that sow body depletion occurs with continued reproductive demands, and that the deficiency onset is exacerbated with age (Nielsen et al., 1979; Chavez, 1985). Sow body composition research (Mahan and Newton, 1995) has demonstrated that sows with heavier litter weaning weights had lower body tissue Se concentrations or a greater loss of several trace minerals than sows of a lower productivity.

Effect on the fetus

When the dam’s diet is low in Se, fetal liver Se has been shown to decline during the gestation period (Piatkowski et al., 1979). Recent research by Hostetler and Kincaid (2004) has additionally demonstrated that oxidative damage occurs in both the sows and fetal liver tissue when a low Se diet is fed to the pregnant animal. This was indicated with higher concentrations of the lipid peroxide malondialdehyde (MDA) and H$_2$O$_2$ in fetal livers but GSH-Px activity of fetal liver was not affected. Their results also demonstrated that liver GSH-Px activity was substantially higher in adult female pigs than in their fetuses. When the Se deficient diet was fed, the oxidative end products accumulated in the fetus. The pig fetus has a relatively low production of GSH-Px, which is apparently inadequate to remove the additional influx of oxidative end products from the dam, thus resulting in an increased concentration in the fetus. The fetus may therefore be unable to synthesize increased amounts of the GSH-Px enzyme to prevent the buildup of these oxidative by-products (i.e., MDA, and H$_2$O$_2$). Although supplemental Se has been shown to reduce the production of the lipid peroxide MDA (Sarada et al., 2002) the fetus is apparently unable to do so. Therefore, the sow’s Se status may be critical to fetal survival by preventing the accumulation of oxidative end-products in conceptus products. Because the amount of Se transferred to the fetus and GSH-Px production is evidently relatively low in the developing fetus, the Se status of the female could be critical to fetal survival. The transfer of Se to the fetus is not only dependent upon the Se status of the female, but also upon the source and level of dietary Se provided to the dam during gestation.

Postnatal effects

When the female is deficient in vitamin E and Se, the neonatal pig may experience various deficiency signs including iron (Fe) toxicosis. Iron toxicity in piglets is a condition where Fe administration to the pig becomes ‘free’ in the circulatory system and the released free Fe exacerates oxidative reactions thus damaging tissue membranes, ultimately causing the death of the pig. Loudenslager et al. (1986) demonstrated that neonatal pigs from sows fed diets low in Se and vitamin E had a poorer antioxidant status than those pigs from sows fed diets fortified with adequate levels of vitamin E and Se. Research with the young chick has also demonstrated that chicks fed a Se-deficient diet developed a necrosis in the muscle tissue suggesting that muscle atrophy may occur in the Se-deficient neonate (Bartholomew et al., 1998).

Because colostrum is high in vitamin E and Se concentrations, it is a major source of these nutrients for the neonatal pig. Consequently, sows with a higher tissue status of these nutrients would be expected to transfer more through the mammary secretions, whereupon the neonate would be better able to cope with the oxidative effects postnatally. Milk is also a good source of Se and vitamin E, but its concentration is somewhat lower compared with colostrum. Consequently, the weaned pig’s vitamin E and Se status will be largely affected by the sow’s body tissue status, what the sow is fed and the amount and form transferred through the placental and mammary tissue to her milk supply. Mahan (1991; 1994) demonstrated that milk Se and vitamin E concentrations normally decline with parity, suggesting that the progeny of older sows would be more prone to the deficiency onset postweaning than the progeny of younger sows. The importance of the pig’s Se status at weaning is demonstrated by the report of Mahan et al. (1974). In that experiment, pigs were obtained from sows that were fed either a non-Se-fortified diet or one
that contained 0.10 ppm Se (selenite), but both pig groups upon weaning were fed a semi-purified diet without supplemental Se. This experiment thus evaluated the post-weaning effects of body tissue Se reserves accumulated during nursing. The results presented in Table 1 demonstrated that by 28 days post-weaning, the majority of pigs from sows fed the non-Se-fortified diet had several classical deficiency signs, while none were present in the pigs from sows fed supplemental Se. Because of the high death loss of pigs from the non-Se-fortified sow diet at 28 days, they were discontinued; but the other treatment group continued on the non Se-fortified postweaning diet. By 56 days these sow Se-supplemented pigs also had clinical signs that the other treatment pig group had demonstrated at 28 days. These results indicate the importance of the Se status of pigs at weaning in preventing the deficiency onset. It would appear that feeding the sow diets that would meet the Se and vitamin E requirement would provide an initial body reserve of these nutrients in the neonatal and weaned pig, and that these reserves could be used during periods of Se need.

Table 1. Selenium carryover from the sow to the progeny.

<table>
<thead>
<tr>
<th>Item</th>
<th>Basal No added Se</th>
<th>Basal + 0.10 ppm Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>28-days post-weaning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. pigs examined</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>White skeletal muscle, %</td>
<td>67</td>
<td>0</td>
</tr>
<tr>
<td>Liver necrosis, %</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Enlarged heart, %</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Gastric ulcers, %</td>
<td>83</td>
<td>0</td>
</tr>
<tr>
<td>56-days post-weaning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. pigs examined</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>White skeletal muscle, %</td>
<td>-</td>
<td>40</td>
</tr>
<tr>
<td>Liver necrosis, %</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Enlarged heart, %</td>
<td>-</td>
<td>40</td>
</tr>
<tr>
<td>Gastric ulcers, %</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>

a Pigs were fed a semi-purified non-Se fortified diet post-weaning
b Pigs were removed from the trial due to sudden deaths.
1Mahan et al., 1974

Effect of inorganic or organic selenium on sow reproduction

Feeding organic Se from an enriched Se yeast source (Sel-Plex®) to reproducing sows as compared with sodium selenite has previously been shown to: 1) enhance the Se status of both the sow and progeny, 2) has resulted in equivalent GSH-Px activities for both Se sources, and 3) the organic Se form has been shown to increase colostrum and milk Se substantially above that of inorganic Se (Mahan and Kim, 1996; Mahan, 2000).

Because Se yeast has now been approved in the US as a dietary Se source for swine (FDA, 2002) as well as in many other countries, its long-term effects on the sow and progeny were evaluated (Mahan, 2004). The results of that study demonstrated that the numbers of total and live pigs born were lower when a non-Se fortified basal diet was fed, but generally there was no significant difference between the two dietary Se sources or the two Se levels evaluated (Figure 1). The number of stillbirths was greater (P<0.05) when inorganic Se was fed (Figure 2). Neonatal pigs having a moderate or severe splay-legged condition also appeared to be greater when the inorganic Se source was fed as compared with organic Se diets, but the responses were not significant (Figure 3).

Serum GSH-Px activity seemed to plateau (P<0.05) at the 0.15 ppm Se level within each parity (Figure 4). Sows fed the basal diet had consistently lower GSH-Px activities within each parity as compared with the two Se sources but the values continued to decline with advancing parity when the basal non-Se-fortified diet was fed. Although sow serum GSH-Px activity also declined by parity in all treatment groups, particularly in parity 3 and 4, the decline was greater when the non-Se-fortified diet was fed.

There was a consistent decline in serum Se after 70 days post-coitum regardless of Se source or Se level fed, suggesting that Se was being transferred to the conceptus products during this phase of reproduction, resulting in the lower circulatory level. The same trends are shown for sow GSH-Px activity where GSH-Px activity was lower at 110 days post-coitum than at day 70, whereupon the values are correspondingly increased by weaning. The latter is undoubtedly reflective of the increased sow feed and Se intake, except for sows fed the non-Se-fortified diet (Figure 5).

Although the Se concentration in colostrum and milk increased more with the feeding of organic Se compared with inorganic Se, there was a decline in milk Se as parity progressed but only when the inorganic Se or the basal non-Se-fortified diets were fed, not when the organic Se diet was provided (Figure 6). These results suggest that the Sel-Plex® organic Se source provided a consistent and relatively constant supply of Se during each lactation, and that it
The role of selenium and Sel-Plex® in sow reproduction

Figure 1. Effect of sow dietary Se source (inorganic selenite or Sel-Plex® organic Se) and level (ppm) on total and live pigs born per litter over the 4-parity period (SEM [total and live]= 0.60, P<0.05). The combination treatment (0.30 ppm Se) contains 0.15 ppm Se from both organic and inorganic Se sources.

Figure 2. Effect of sow dietary Se source (inorganic selenite or Sel-Plex® organic Se) and level (ppm) on the average number of stillbirths per litter over the 4-parity period (SEM = 0.05, P<0.05). The combination treatment (0.30 ppm Se) contains 0.15 ppm Se from both organic and inorganic Se sources.

appeared to be readily incorporated into milk proteins. This also suggests that an increasing amount of Se would be consumed by the nursing pigs during each parity when sows are fed organic Se, thus enhancing the pig’s Se status at weaning.

Consequently, pigs that nursed sows fed the non-Se-fortified diet had lower serum Se concentrations at weaning as compared with pigs from sows fed Se-fortified diets (Figure 7). Pigs from sows fed the non-Se-fortified diet had a higher serum Se in parity 1 but the concentrations declined with advancing parity. This suggests that sow depletion of Se was a contributing factor in milk Se concentration over time. Weanling pig serum Se was greater (P<0.01)
when sows were fed Sel-Plex® as compared to inorganic Se, responses consistent with previous reports (Mahan, 2000).

Pig serum GSH-Px activity at weaning was also lower (P<0.01) when sows were fed the basal non-Se-fortified diet than when sows were fed the Se-fortified diets (Figure 8). Pig serum GSH-Px activity was increased (P<0.05) as sow dietary Se level increased to 0.30 ppm Se from both dietary Se sources. These serum GSH-Px activities did not differ significantly between basal and combined treatment groups.
The role of selenium and Sel-Plex® in sow reproduction

between the two Se sources nor was the interaction between the factors significant. There was an apparent decline in pig serum GSH-Px activity as parity progressed in all treatment groups, the reason for which is unclear.

Sow tissue Se concentrations were greater at the end of the 4-parity period when organic Se was fed, responses consistent with previous reports (Mahan and Kim, 1996). Because of interest in using hair as an analytical tool for evaluating sow Se status, hair Se content was analyzed at the time of weaning for each treatment group. Hair Se content was greater when sows were fed increasing levels of organic (P<0.01) or inorganic Se (P<0.05), but the Se

**Figure 5.** Effect of Se source (inorganic selenite or Sel-Plex® organic Se), and level (ppm) on sow serum GSH-Px activity at various stages of gestation and at weaning (SEM = 0.03, interaction of Se source x level x production phase P<0.01). The combination treatment (0.30 ppm Se) contains 0.15 ppm Se from both organic and inorganic Se sources.

**Figure 6.** Effect of parity, dietary Se source (inorganic selenite or Sel-Plex® organic Se), and level (ppm) on milk Se concentrations at weaning (SEM = 0.003; Parity (P<0.01), interaction of parity by Se treatment (P<0.01)). The combination treatment (0.30 ppm Se) contains 0.15 ppm Se from both organic and inorganic Se sources.
concentration was substantially greater when the organic Se source was fed (Figure 9). When the combination of Se sources were fed, sow hair Se concentrations were generally similar to the group fed 0.15 ppm organic Se. Hair Se concentration when sows were fed 0.30 ppm Se from Sel-Plex® seemed to be relatively constant over the 4-parity period, whereas the other treatment groups showed a small but consistent increase by parity but this response was not significant. The sulfur (S) in the amino acid cysteine in swine hair is thought to be greater (i.e. >13%) than in other tissues (Mahan and Shields, 1998). Because of the substitution of S with Se in these amino acids (i.e., methionine or seleno-
The role of selenium and Sel-Plex® in sow reproduction

methionine) the resulting hair Se had a greater relative Se concentration when selenomethionine was in a higher concentration in the diet. Because these amino acids can substitute for each other in the tissues, the results suggest that selenomethionine or selenocysteine would be a major contributor to hair Se when present in the animal’s diet and thus in its circulatory system. Se was probably present in a higher relative concentration when the organic Se source was fed at the 0.30 ppm Se level than when inorganic Se was the major Se source or when the lower (i.e., 0.15 ppm) organic Se level was fed. The present experiment suggests that the Se content of sow hair appears to largely reflect the dietary source and level of organic Se that was fed to the sow, and does not reflect the sow’s Se status.

What does all of this mean?

The results of several experiments show that the sow may experience hyposelenosis, particularly if an inadequately fortified diet is fed. A hyposelenosis condition can affect her reproductive capability, and it can influence her body’s Se status and Se status of her progeny. Sow tissue can become depleted of Se over time, and sows of higher productivities and greater age are more prone to the deficiency occurrence. Inorganic Se (selenite) was critical in the initial discovery that Se had a vital role in swine production, but because it is not as well retained by the pig and less is transferred to the fetus and milk products in the adult female, the organic Se form might be superior over prolonged reproductive periods. Although inorganic Se is effective in producing the selenoenzymes necessary for antioxidant protection, organic Se seems to be equally effective. The use of organic Se is beginning to show advantages as a source of Se for the sow over that of inorganic Se. Feeding organic or inorganic Se at 0.15 ppm appears to optimize reproductive performance, but the higher level (i.e., 0.30 ppm Se) of the organic Se source will more greatly increase the transfer of Se to the fetus and milk products than the lower 0.15 ppm Se level or the inorganic Se source.

References
FDA. 1974. Food additives permitted in feed and

![Figure 9. Effect of parity, dietary Se source (inorganic selenite or Sel-Plex® organic Se), and level (ppm) on sow hair Se concentrations at weaning (SEM = 0.028; Parity (P<0.01)). The combination treatment (0.30 ppm Se) contains both organic and inorganic Se sources.](image-url)
The role of selenium and Sel-Plex® in sow reproduction
Adding value to pork for producers and consumers: enhancing omega-3 DHA and selenium content of meat

PAUL PENNY

JSR Genetics Ltd, Southburn, Driffield, United Kingdom

Introduction

Pork is possibly the most versatile raw meat product compared to chicken, beef, turkey and lamb. By utilising sophisticated processing techniques it can be easily transformed and presented across a highly varied range of quality products such as fresh pork, spare ribs, specialist hams, bacon, sausages and salamis. Developing and marketing new pork products is becoming an increasingly competitive activity due to the globalisation of the pig industry and the homogenisation of the markets. Consumers are becoming more particular regarding what they buy; they are not only concerned with the welfare of the animal but the health of their own diets and what nutrients the presented product can offer over and above alternative protein sources.

Meat consumption in the EU and USA (per capita consumption) is 44 kg and 30 kg, respectively, with pig meat consumption being higher than all the other meats. The present and future forecast projection for the EU is 45 kg/hd (Table 1), although this is extremely static when viewed over a 10-year period (Pig Progress, 2001).

For pork to continue to compete successfully in the international and national meat protein market, it must demonstrate an unquestionable stance on safety, quality, convenience, healthiness and price. Recent data compiled by a leading retailer in the UK (Figure 1) clearly showed that pork was seriously failing to attract the contemporary consumer (Smith, 2003). Although this may be specific to the UK market, it should be seen as a warning and acted upon accordingly. To continually improve the image of pig meat and hence consumption it is necessary not only for the retailers but the producers and processors to portray the correct image to the consumer. There is an ever-increasing demand by the public for varied diets containing even safer and healthier foods. Providing the consumer with new and exciting products that can deliver consistent quality and complement an ever-growing need for a healthy diet and lifestyle should be fully embraced. The successful company of the future must create wealth within a very challenging environment, requiring the ability to deal with confusion, unanticipated market movements and rapid change. It is therefore extremely important to develop an entire pork chain that is knowledge-based. Networking is required to provide synergistic benefits through exchange, association and mutuality enabling greater achievements than the individual working alone.

The need for identifying a positive way to promote and re-position pork as a healthy, tasty and nutritious product is of paramount importance and was the key driving force behind the development of Vitapork™. This new product delivers substantial amounts of essential omega-3 DHA polyunsaturated fatty acids together with major antioxidants (selenium and vitamin E). It is believed that Vitapork™ has all the necessary characteristics and final product benefits to fully complement the consumer’s growing appetite for healthy nutritious food.

Table 1. Per capita pig meat consumption projections in the EU, 1999-2008.

<table>
<thead>
<tr>
<th>Year</th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
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<tr>
<td>Consumption, kg/head</td>
<td>44.4</td>
<td>43.4</td>
<td>44.2</td>
<td>44.7</td>
<td>44.9</td>
<td>44.7</td>
<td>45.2</td>
<td>45.8</td>
<td>45.8</td>
<td>46.1</td>
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</table>
Consumer behaviour

Meat and meat products are important components in the diets of developed countries and their consumption is affected by various factors such as preparation time, cooking convenience and overall enjoyment. There is also a shift in the type of meat product consumed from fresh to processed products. Consumer needs are constantly changing; and this will be enhanced in the future through increasing disposable income. The consumer has a vast choice of products and must balance lifestyle activities against the need to consume food. An understanding will be required of how product presentation must respond to these trends. In the next 10-20 years a higher proportion of the population will be over 45 years of age, which means that this group of consumers will have a larger influence on pork consumption than the younger generation. The traditions and habits of the consumer will gradually change largely due to the consumers’ lifestyles. Sitting around a table as a family will occur less frequently and therefore eating out, which involves quick, on-the-go consumption of convenience products will become a prominent feature of food consumption.

Over the last 50 years there has been a significant decline in the consumption of omega-3 (n-3) polyunsaturated fats. The main reason for this reduction of omega-3 DHA consumption is the dramatic change in eating habits and more particularly a change in the type of foods consumed. Those food products containing high levels of omega-3 DHA fats such as eggs, offal and oily fish have lost their place in the modern diet. Leading nutritional experts and organisations like the Foods Standards Agency within the UK are now actively promoting the fact that consumers should make every effort to increase their daily intake of omega-3 polyunsaturates.

Omega-3 fatty acids

Fatty acids are the major building blocks of all lipids. Their division into two groups, non-essential, i.e. need not be supplied in the diet, and essential, i.e. must be supplied in the diet, has been known since the early 1930s. Fatty acids not only serve as structural components of all cells but also take part in and are of paramount importance to cellular metabolism. This is particularly so for the essential fatty acids; and ever since their discovery an ever increasing number of roles for them have been found ranging from basal metabolism to the maintenance of health and well-being (BNF, 1992) (Table 2).

The essential fatty acids can be divided into the n-6 group based on linoleic acid (LA) and its longer chain, more unsaturated derivatives, and the n-3 group, which is based on α-linolenic acid (LNA) and its derivatives. Until relatively recently, the balance of nutritional interest was heavily weighted in favour of the n-6 polyunsaturates and their role in health promotion; the imbalance with the n-3 group
being of an order of magnitude and primarily due to the consumption of cooking oils and margarines. The extent of n-6 polyunsaturate consumption was such that adverse effects on health were a distinct possibility. Furthermore a comprehensive involvement for the n-3 polyunsaturates in a wide range of health issues and disease prevention was becoming increasingly clear (BNF, 1992). As a result, a far more balanced nutritional strategy in the provision of the two groups of polyunsaturates is now recognised as essential. With the recognition that the groups of polyunsaturates each have their own distinctive metabolic roles and involvements in health promotion, it is also recommended that emphasis on the polyunsaturated:saturated fatty acid (P:S) ratio as a measure of dietary acceptability be reduced and that the importance of the separate n-6 and n-3 fatty acids be recognised through an appropriate total n-6:total n-3 ratio or even an LA:LNA ratio (BNF, 1992; Bruckner, 1992). Furthermore, consumption of n-3 acids should be increased with an aim to achieving an n-6:n-3 ratio of 6:1. The major fatty acid of the omega-3 series, which is seen as being the most important for the body, is docosahexaenoic acid (DHA).

Many investigations have looked at the effect of increasing the levels of polyunsaturated fatty acids in pig feed, which has been noted to increase carcass fat content. Feeding linseed oil has been the primary route taken to achieve this natural modification of the fat and muscle tissue. Addition of linseed oil can successfully increase carcass level of LNA (C18:3), the first fatty acid in the omega–3 family. However, this method of dietary modification has minimal effect on the most important and highly beneficial omega-3 long chain polyunsaturated fatty acids (LCPUFA), eicosapentaenoic (EPA, C22:5) and docosahexaenoic acid (DHA, C22:6), in terms of human health and well-being. The reason for this lack of improvement relates to the complicated biochemical process of desaturation and chain elongation, which is required to convert LNA into EPA and DHA. Efficiency of conversion from LNA to EPA and DHA within the pig is no more than 5%, about the same as in the human (Emken et al., 1994). Therefore, the only way of significantly increasing the key LCPUFA, EPA and DHA, in the carcass is by incorporating them directly into the diet.

Antioxidant requirements: selenium and vitamin E

Unsaturated fatty acids are particularly susceptible to oxidation; which is substantially increased in highly unsaturated molecules such as DHA through a cascade of oxidative events. Oxidation is brought about by the action of oxygen free radicals, which naturally occur and potentially accumulate in living and post-mortem cells, on the highly unsaturated lipids in the cell membrane and contents (Burton, 1994). The breakdown can be further enhanced by post-mortem handling that facilitates interaction between pro-oxidant factors and the unsaturated lipids. The accumulated presence of the oxidised lipid metabolites not only poses a potential cytotoxic threat but also reduces product acceptability due to off-flavours, rancidity and malodorous compounds.

However, cells have an array of natural defense mechanisms against free radical formation and lipid oxidative damage (Frust, 1996). These consist of concerted enzyme systems that eliminate free radicals.
and a selection of naturally occurring vitamins and synthesized products, both fat and water soluble, to deter the cascade of events that leads to radical accumulation. Important amongst the former are the enzymes superoxide dismutase, catalase and glutathione peroxidase; and amongst the latter vitamins A, C and E, ubiquinones and a range of natural plant metabolites. The whole sequence of events is further affected by a selection of pro-and antioxidant multivalent metal ions e.g. iron, copper, selenium, zinc. With an increasing interest in the antioxidant defence. The availability and usefulness of selenium from the diet is dependent on the form in which it occurs. This can be either natural selenoamino acids, which are found in plants or inorganic sources like sodium selenite that are usually present in standard trace mineral premixes.

The major benefit of selenium is as an antioxidant enzyme cofactor, preventing damage to cells by oxidation. As part of the glutathione peroxidase molecule and other antioxidant selenoenzymes, selenium status plays a major role in the body’s antioxidant defence. The availability and usefulness of selenium from the diet is dependent on the form in which it occurs. This can be either natural selenoamino acids, which are found in plants or inorganic sources like sodium selenite that are usually present in standard trace mineral premixes.

Selenomethionine, the primary form present within Sel-Plex® provides added advantages over the sodium selenite. It enables both increased availability and tissue selenium reserves that can be used quickly and effectively during increased demand. The selenium accumulated in body proteinaceous tissues when selenoamino acids are included in the diet provides an opportunity for consumers of such animal products to benefit through increased intake of selenium.

Vitamin E is the main fat-soluble antioxidant in cell membranes. It is stored in fatty tissue, liver and muscle. It can neutralise free radicals and help stop them from reacting further. In this way it acts like a protective shield around each cell, reducing tissue damage. This beneficial action of being able to slow down oxidation clearly identifies vitamin E as an important protectant for improving the quality of fresh, processed and frozen meat products.

Vitamin E can stabilise the colour of red meat, but most importantly it stops fat turning rancid and helps alleviate off tastes and odours. The role and function of vitamin E become absolutely paramount when modifications are made to the fatty acid composition of the fat and lean tissue. Increasing the polyunsaturated content of the diet and hence the number of double bonds in the fat and meat substantially intensifies the susceptibility of the product to free radical attack. To fully ensure optimal benefits, animals must be fed significant amounts of vitamin E above those normally found in standard production diets. An achievable target for maximising superior product quality would be 5-6 mg vitamin E (as α-tocopherol) per kilogram of muscle tissue. It is very difficult to obtain the same effects by treating meat products during processing.

**Vitapork™ ‘Choose the healthy option’**

Vitapork™ is an innovative approach to enhancing the ‘healthiness’ and nutritional value of pork. It is a ‘brand’ of new lean and healthy meat that can be utilised across a range of final product formats.

The object of the Vitapork™ program was to develop a new approach toward enhancing the healthiness of pork. The strategy was to increase the most important LCPUFA, EPA and DHA, and obtain the recommended n-6:n-3 ratio without compromising the physical and organoleptic properties of the carcass. Accepted understanding to date has suggested that increasing the dietary concentration of linoleic acid (C18:2) n-6, and particularly the carcass concentration, e.g. above 15% of the total fat, leads to a substantial softening of the depot fat, thereby producing carcasses with highly unacceptable processing properties which fail to satisfy consumer requirements (Whittington et al., 1986). However it is now possible to overcome this negative outcome by utilising a combination of oil products in the diet. When elevating the PUFA level, antioxidant protection becomes critical in order to provide the necessary assurance against lipid oxidation. To fulfil this need a combined selenium (Sel-Plex®) and vitamin E supplement was paramount.

The extensive amount of literature highlighting the health benefits of consuming these specific LCPUFA and the additional benefits of consuming elevated concentrations of selenium and vitamin E was one of the main reasons for developing and producing the Vitapork™ product. The need for identifying a positive way to promote and re-position pork as a healthy, nutritious product in terms of PUFA levels without compromising physical properties and organoleptic acceptability was also a significant driving force.

A series of controlled experiments and extensive field studies were undertaken to investigate various diet compositions and feeding time periods (Table 3). In association were extensive carcass assessments
(Table 4). The total PUFA level in the fat tissue increased from 18% to 34% in the Vitapork™ product, which would be equivalent to the profile of good quality table margarine.

Table 3. Percentages of DHA in lean meat and fat following dietary supplementation for 2-6 weeks.

<table>
<thead>
<tr>
<th>Weeks of supplementation</th>
<th>Lean meat</th>
<th>Fat</th>
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<tbody>
<tr>
<td>2</td>
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<td>0.25</td>
</tr>
<tr>
<td>4</td>
<td>0.80</td>
<td>0.40</td>
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<tr>
<td>Vitapork™ - 5</td>
<td>0.98</td>
<td>0.63</td>
</tr>
<tr>
<td>6</td>
<td>1.01</td>
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</tbody>
</table>

Table 4. Carcass enhancement following a 5-week supplementation period.

<table>
<thead>
<tr>
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<th>Standard</th>
<th>Vitapork™</th>
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</thead>
<tbody>
<tr>
<td>PUFA, % of fat</td>
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<td>34</td>
</tr>
<tr>
<td>n-6:n-3</td>
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</tbody>
</table>

The Vitapork™ technology involves a diet containing very specific LCPUFA (EPA and DHA) in combination with a significant level of LA. To demonstrate the robustness of the Vitapork™ feeding protocol, three separate commercial units each having different housing facilities were used during the finishing period. In addition, three genotypes were investigated to show that the technology can be broadly utilized. All animals were weighed at the beginning and end of the supplemental period (Table 5).

Table 5. Growth and carcass response following the five-week supplemental period.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vitapork™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype A - n=16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start weight, kg</td>
<td>61.5</td>
<td>62.5</td>
</tr>
<tr>
<td>End weight, kg</td>
<td>88.5</td>
<td>90.0</td>
</tr>
<tr>
<td>Daily gain, g/day</td>
<td>77.1</td>
<td>78.5</td>
</tr>
<tr>
<td>P2, mm</td>
<td>9.3</td>
<td>9.7</td>
</tr>
<tr>
<td>Genotype B - n=80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start weight, kg</td>
<td>64.3</td>
<td>64.8</td>
</tr>
<tr>
<td>End weight, kg</td>
<td>93.5</td>
<td>92.9</td>
</tr>
<tr>
<td>Daily gain, g/day</td>
<td>83.4</td>
<td>82.2</td>
</tr>
<tr>
<td>P2, mm</td>
<td>10.4</td>
<td>10.3</td>
</tr>
<tr>
<td>Genotype C - n=40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start weight, kg</td>
<td>64.0</td>
<td>64.1</td>
</tr>
<tr>
<td>End weight, kg</td>
<td>98.9</td>
<td>98.4</td>
</tr>
<tr>
<td>Daily gain, g/day</td>
<td>99.2</td>
<td>98.0</td>
</tr>
<tr>
<td>P2, mm</td>
<td>11.0</td>
<td>11.2</td>
</tr>
</tbody>
</table>

The carcass produced by the Vitapork™ diet provided both an enhanced LCPUFA content and improved n-6:n-3 ratio, but equally importantly provided a very acceptable carcass in terms of processing and final product formats. The diet formulation utilises key active ingredients, such as high-DHA tuna oil, soya oil, organic selenium (Sel-Plex®) and vitamin E.

The improvements from implementing the 5 weeks pre-slaughter feeding protocol are due to an increase of 300% in DHA and 68% for LA compared to standard product (Figures 2 and 3). The ability to significantly increase both carcass EPA and DHA and more specifically LA, whilst at the same time maintaining overall carcass acceptability, goes against previous knowledge regarding fatty acid enhancement of the carcass. The final product also has the added benefit of being fully traceable and checked for authenticity, meaning that the carcass can be checked and easily identified by undertaking a simple laboratory analysis.

This exciting opportunity of carcass enhancement has the ability to make pork the first choice option when purchasing meat. Vitapork™ truly delivers multi-functional meat, which provides the health conscious consumer with a high quality product containing enhanced levels of omega-3 DHA, selenium and vitamin E (Table 6).

Table 6. Characteristics of Vitapork™.

- Vitapork™ can provide 50 mg of DHA per 100 g of pork
- Vitapork™ is significantly differentiated from standard pork
- Vitapork™ delivers clear and identifiable benefits consistently and provides a novel premium meat product.
- Vitapork™ complements consumer needs for quality, healthy and convenient products.

Summary

The secret to producing good quality food is to identify the consumer’s quality criteria and act appropriately to meet them. Pig meat will need to be marketed to the consumer based on production using natural ingredients, and it will be highly beneficial if products can be traced from production through to manufacturing.

The consumer must also be able to recognise these branded meat products on the shelf and feel comfortable with the endorsement. Regaining the required image and restoring consumer trust requires a co-ordinated effort and a vision shared by all participants in the food chain including farm suppliers, service providers, farmers, meat processors,
Adding value to pork for producers and consumers

Information needs to flow along the chain in both directions, which can boost efficiency and allow all to benefit from the added value achieved.

References


Reducing the environmental impact of swine production through nutritional means

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Introduction

Livestock production is becoming more concentrated in many parts of the world and pork production is no exception. This is particularly evident when examining a recent Canadian report (Saskatchewan Agriculture Food and Rural Revitalization, Statistics Canada, 2003) showing the pig densities per square mile in different countries of the world (Table 1). More and more people residing in rural areas are not accustomed to practices associated with crop and livestock production. Certainly, producers want to remain profitable in order to continue in the pork business in the future. However, most if not all pork producers also maintain the goal of producing pork in a socially acceptable and environmentally sound manner (Coffey, 1999). The actual numbers of pigs produced has not changed drastically since 1900 (Table 2). In fact, the environmental impact of pork production is likely less today than 100 years ago when examined on a per pig basis. However, the same number of pigs is being produced on fewer farms, which increases the environmental risk in a concentrated area (Table 2). In 2000, 51% of the pigs marketed were coming from operations that marketed more than 50,000 pigs annually (National Pork Board, 2002).

The general public is increasingly concerned with the environmental circumstances under which food is produced. Public concerns center around soil (accumulation and runoff of minerals from land where manure is applied), water (surface and ground water), and air quality environmental issues. Federal, state, and local laws have and will continue to impose regulations designed to address the environmental concerns associated with crop and livestock production. Jongbloed and Lenis (1998) reported that many countries have limited the use of livestock

<table>
<thead>
<tr>
<th>Country or region</th>
<th>Pig inventory</th>
<th>Pigs per acre (hectare)</th>
<th>Pigs per sq mile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>25,958,000</td>
<td>0.89 (2.20)</td>
<td>569.1</td>
</tr>
<tr>
<td>Spain</td>
<td>23,858,000</td>
<td>0.74 (1.83)</td>
<td>474.6</td>
</tr>
<tr>
<td>France</td>
<td>15,290,000</td>
<td>0.34 (0.83)</td>
<td>214.7</td>
</tr>
<tr>
<td>Netherlands</td>
<td>13,000,000</td>
<td>5.81 (14.36)</td>
<td>3,720.6</td>
</tr>
<tr>
<td>Denmark</td>
<td>12,990,000</td>
<td>2.29 (5.67)</td>
<td>1,467.9</td>
</tr>
<tr>
<td>Belgium</td>
<td>6,851,000</td>
<td>3.29 (8.14)</td>
<td>2,107.4</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>5,588,000</td>
<td>0.40 (0.99)</td>
<td>256.1</td>
</tr>
<tr>
<td>Asia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>9,612,000</td>
<td>0.88 (2.16)</td>
<td>560.1</td>
</tr>
<tr>
<td>China</td>
<td>464,695,000</td>
<td>1.31 (3.24)</td>
<td>838.0</td>
</tr>
<tr>
<td>United States</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iowa</td>
<td>15,000,000</td>
<td>0.56 (1.38)</td>
<td>357.9</td>
</tr>
<tr>
<td>North Carolina</td>
<td>9,600,000</td>
<td>1.71 (4.23)</td>
<td>1,095.2</td>
</tr>
<tr>
<td>Minnesota</td>
<td>6,100,000</td>
<td>0.28 (0.70)</td>
<td>181.7</td>
</tr>
<tr>
<td>Illinois</td>
<td>3,950,000</td>
<td>0.16 (0.40)</td>
<td>104.6</td>
</tr>
<tr>
<td>Indiana</td>
<td>3,100,000</td>
<td>0.23 (0.57)</td>
<td>148.4</td>
</tr>
<tr>
<td>Canada</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quebec</td>
<td>4,280,000</td>
<td>0.93 (2.31)</td>
<td>597.7</td>
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<tr>
<td>Ontario</td>
<td>3,700,000</td>
<td>0.41 (1.01)</td>
<td>261.0</td>
</tr>
<tr>
<td>Manitoba</td>
<td>2,750,000</td>
<td>0.22 (0.55)</td>
<td>143.3</td>
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<tr>
<td>Alberta</td>
<td>2,100,000</td>
<td>0.08 (0.19)</td>
<td>49.6</td>
</tr>
<tr>
<td>Saskatchewan</td>
<td>1,211,000</td>
<td>0.03 (0.07)</td>
<td>16.9</td>
</tr>
</tbody>
</table>

1Adapted From Saskatchewan Agriculture Food and Rural Revitalization, Statistics Canada.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of hogs</th>
<th>Number of operations</th>
<th>Pigs per operation</th>
<th>Average retail meat yield per carcass</th>
</tr>
</thead>
<tbody>
<tr>
<td>1900</td>
<td>62,879,000</td>
<td>4,355,989</td>
<td>14.5</td>
<td></td>
</tr>
<tr>
<td>1920</td>
<td>59,350,000</td>
<td>4,852,430</td>
<td>12.2</td>
<td></td>
</tr>
<tr>
<td>1940</td>
<td>34,040,000</td>
<td>3,767,875</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>1960</td>
<td>59,030,000</td>
<td>1,848,784</td>
<td>31.9</td>
<td>124</td>
</tr>
<tr>
<td>1980</td>
<td>67,353,000</td>
<td>670,350</td>
<td>100.5</td>
<td>133</td>
</tr>
<tr>
<td>2000</td>
<td>59,073,000</td>
<td>86,360</td>
<td>684.0</td>
<td>151</td>
</tr>
</tbody>
</table>

Reducing the environmental impact of swine production

Livestock producers must implement these regulations in times when margin or profit per animal sold continues to decline (National Pork Board, 2002). The combination of laws, neighbor relations, and economic concerns along with the concentration of pork and other livestock production brings about challenges for producers. The objective of this paper is to review the nutritional methods to reduce the environmental impact of swine production.

Feeding practices and excretions

Food animals convert feed nutrients into various animal products including meat, milk, eggs, wool, hides, draft power, feces, urine, and gases (fermentation and respiration). The amounts of nutrients required depend on the animal’s genetic capacity for each of these products under a variety of environmental conditions. Today, producers are able to control what the pig eats because modern swine systems utilize confinement facilities in a very large proportion of the industry. Relatively few animals are raised in outdoor systems where pigs can forage for food other than the diet provided by the producer. As a result, manure concentrations of elemental nutrients in swine are direct functions of nutrient inputs in the diet (Van Horn and Powers, 2004). Until recently, livestock producers were generally concerned only with maximizing food animal production. However, when including livestock production into a whole farm plan, often maximization of production output does not make economic sense. A better approach would be to develop a farm plan that optimizes livestock production for the operation as a whole.

There are several ways that diets fed to pigs can be altered to reduce the nutrients excreted and the odors produced from pork production thereby reducing environmental impact. Those nutrients that are generally considered to cause the most environmental concern are phosphorus, potassium, carbon dioxide, methane, ammonia and the nitrogenous compounds nitrite/nitrate, and nitrous oxide. Kornegay and Harper (1997) outlined means to accomplish a reduction in the excretion of the components of manure that pose the greatest environmental concern:

1) Improve feed efficiency.
2) Know the pig's nutrient requirements and nutrient composition of feedstuffs.
3) Avoid over-formulating diets.
4) Feed for optimal growth rather than maximum.
5) Use of crystalline amino acids and high quality protein sources.
6) Improve mineral availability and/or use sources with high bioavailability.
7) Feed to the pigs exact needs by stage of growth and gender.
8) Reduce feed wastage.

It is clear that not all dietary nutrients ingested are absorbed, utilized, and retained by the pig. Kornegay and Harper (1997) outlined the percentages of various nutrients digested and retained by grow–finish pigs (Table 3). The percentage of nutrient digested ranged from 5% to 88% and the percentage of nutrient retained ranged from 10% to 70%. This clearly points out that it is not possible for 100% utilization of nutrients by pigs or other livestock. However, several factors can influence the quantity of each nutrient retained or excreted (Kornegay and Verstegen, 2001). These factors include age, class, and nutritional status of the pig; source, quality, amount, and proportion of nutrients provided in the diet; processing methods; and environmental influences (Kornegay and Verstegen, 2001).

Table 3. Nutrient digestion and retention by growing – finishing swine.¹

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Digested</th>
<th>Retained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>75-88</td>
<td>40-50</td>
</tr>
<tr>
<td>Calcium</td>
<td>40-75</td>
<td>25-70</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>20-70</td>
<td>20-60</td>
</tr>
<tr>
<td>Magnesium</td>
<td>20-45</td>
<td>15-38</td>
</tr>
<tr>
<td>Sodium</td>
<td>35-70</td>
<td>13-25</td>
</tr>
<tr>
<td>Potassium</td>
<td>60-80</td>
<td>10-20</td>
</tr>
<tr>
<td>Zinc</td>
<td>10-40</td>
<td>Similar to digested</td>
</tr>
<tr>
<td>Copper</td>
<td>10-25</td>
<td>Similar to digested</td>
</tr>
<tr>
<td>Iron</td>
<td>5-35</td>
<td>Similar to digested</td>
</tr>
<tr>
<td>Manganese</td>
<td>8-40</td>
<td>Similar to digested</td>
</tr>
</tbody>
</table>

¹Adapted from Kornegay and Harper, 1997

The nutrient requirements of swine are well documented (NRC, 1998). However, these requirements are based on research conducted on pigs that do not have the genetic capacity for lean accretion shown in some modern pig lines. Additionally, as shown in Table 3, many nutrients have a range of...
digestibility and retention values that are dependent on feed quality, processing, and other factors. For these reasons, many nutritionists and feed suppliers commonly recommend nutrient levels that include safety margins, resulting in diets that are formulated to exceed NRC requirements. Nutritionists commonly over-fortify diets to ensure that the pig has a supply of nutrients that allows the animal to meet its genetic capacity for growth under a variety of conditions.

There are two requirements needed to minimize the amount of nutrients excreted and maximize the nutrient quantities digested and retained. First, the pig’s nutritional needs must be accurately known for every genetic type, size and gender of pig, under all environmental conditions. Second, the bioavailability of each nutrient from every feed source must be known. Unfortunately, neither is known precisely at the present time.

**NITROGEN**

Feeding diets that more closely provide the pig with the ideal amino acid pattern has been shown to reduce the amount of nitrogen excreted. Traditionally, corn–soybean meal diets have an excess of some amino acids in order that the requirement of the most limiting amino acid, lysine, is met. However, excess protein can be reduced substantially through balancing diets using a reduced amount of soybean meal and including crystalline amino acids (Carter et al., 1996). By including the crystalline amino acids lysine, threonine, and tryptophan, nitrogen excretion was reduced by nearly 30% (Bridges et al., 1995). Similarly, feeding diets that contain protein sources that are highly digestible reduces the amount of each specific nutrient excreted when the ration is balanced based upon bioavailability of the most limiting nutrient. Kerr and Easter (1995) reviewed several articles and estimated that for each percentage point reduction in crude protein obtained by balancing the diet using crystalline amino acids, nitrogen excretion is reduced by approximately 8 percentage points. These results have been more recently supported by those of Sutton et al. (1998), Otto et al. (2003), and Shriver et al. (2003).

**PHOSPHORUS AND OTHER MINERALS**

Poor availability of minerals can pose a greater environmental problem. Of particular concern are the heavy metals and other minerals. Table 3 shows the digestibility and retention values of several minerals. It is clear that mineral bioavailability can vary substantially. Awareness of this variation causes many nutritionists to over-fortify minerals in swine diets, the excess of which is excreted. Phosphorus, zinc, and copper, specifically, have received attention (Moore et al., 2001). Phosphorus builds up in soils where manure is applied and can pose runoff problems, which in turn contributes to eutrophication of surface waters. Land application of minerals has become an increasing environmental concern and is now regulated in many livestock-producing regions. As previously indicated, phosphorus is one of the minerals fed in relatively high amounts due to its poor availability and its importance in various metabolic functions in the pig. The relatively high inclusion rates are due to a high proportion of the phosphorus in feedstuffs commonly fed to pigs being in an unavailable form (phytate). Cromwell and Coffey (1991) reported that as much as two thirds of the phosphorus in corn-soybean meal swine diets is in phytate form.

One way to increase phosphorus bioavailability is by including the enzyme phytase in swine rations. This enzyme releases a portion of the phytate making it available to the pig, which in turn reduces the need for the inclusion of inorganic phosphorus in the diet. Figure 1, adapted from Kornegay et al. (1998) shows the percentage reduction in phosphorus excreted when phytase is supplied in swine diets with reduced inorganic phosphorus inclusion. McMullen and Karsten (2001) reported that the inclusion of phytase combined with reduced inorganic phosphorus resulted in approximately 20% reduction in phosphorus excreted. Additionally, this study also consistently demonstrated improved feed efficiency of pigs fed diets containing phytase. Pierce et al. (2001) demonstrated that phytase did not improve feed efficiency, but did tend to improve rate of gain. Lyons and Cole (2000) summarized several studies, showing that a 0 to 0.20 percentage decrease in dietary phosphorus combined with the use of phytase reduced phosphorus excretion 23 to 53%.

There are numerous other studies that demonstrate improved grow–finish swine performance when phytase is added to diets. This can have positive environmental impact several ways. First, the direct effect of reduced phosphorus in the manure of pigs fed diets containing phytase and reduced amounts of added inorganic phosphorus. Second, if feed efficiency is improved, the same number of pigs would excrete less nutrients as it would take less feed...
Reducing the environmental impact of swine production

to produce the same amount of gain. Third, if rate of gain is improved and pigs are sold at the same weights, the pigs would be on feed for fewer days thus reducing the amount of feed needed for maintenance and reducing the amount of nutrients excreted. Lastly, it has been documented that phytate increases the dietary requirement for zinc. If phytase is utilized and the phytate thereby hydrolyzed, then the amount of dietary zinc can be reduced, thereby reducing the potential environmental impact of excess dietary zinc.

Trace minerals are becoming an environmental concern in areas that rely on repeated land application of manure. Metals accumulate in the soil when fed in excess to pigs. These minerals pose problems, as they can adversely affect the growth of aquatic organisms or even become toxic to some fish like blue gill, minnows, and rainbow trout (Davis, 1974). Additionally, certain metals tend to accumulate in the food chain and pose a toxicity problem to sensitive animal species, such as sheep. Use of minerals with high bioavailability and phase feeding to meet physiological needs can substantially reduce mineral amounts excreted and the environmental risk of soil-applied manure.

Pierce et al. (2001) demonstrated that swine fed diets containing organic minerals (Bioplex™) had similar performance to those fed inorganic sources, but had a 46% decrease in fecal copper concentrations (Figure 2). Leeson (2003) found that using trace minerals with greater bioavailability (Bioplex™ trace mineral) did not affect body weight gain and had little effect on feed efficiency of broilers even when fed at 20% of the inorganic trace mineral level. The authors are conducting a similar study with swine although the lowest level of the Bioplex™ trace mineral inclusion is not as low as that used in the Leeson (2003) broiler study.

PHASE FEEDING

Another way to reduce the amount of nutrients excreted is to meet the pig’s requirements for growth more precisely. This can be accomplished many ways. It is well known that different genetic lines have different capacities for lean growth deposition (Schinckel and deLange, 1996; Thompson et al., 1996). Producers should know the genetic capacity of their pigs for growth, lean deposition, milk production, etc. in order to match diet nutrient fortification with level of production. Additionally, it is well known that the requirements of nursery and grow–finish swine change as body weight and feed intake increase. The Iowa State Life Cycle Swine Nutrition Guide (Holden et al., 1996) demonstrates how diets can be modified for pigs of different body weights. Similarly, it has been shown (Schinckel et al., 1996) and accepted by the industry that the
nutrient requirements of barrows and gilts are different and indeed recommendations for feeding them are different (Holden et al., 1996). The most appropriate way to meet the nutrient requirements of pigs and minimize the excreted nutrients would be to change the diet on a daily basis. However, this is not practical and in most cases not more than 4-6 diet changes are recommended for barrows and gilts throughout the grow–finish phase (Holden et al., 1996). The majority of US pork producers are implementing some form of phase feeding and a large portion implement split sex feeding. In this manner, pigs of different genders and live weights can be fed diets that more closely meet their needs. This type of feeding method reduces the environmental impact to a larger degree when compared to a feeding method that utilizes a diet that meets the needs of a younger, smaller animal and is fed throughout the full grow–finish period.

Henry and Dourmad (1993) demonstrated that nitrogen output could be reduced when grow-finish pigs were fed two or three diets rather than a single phase feeding regimen (Table 4). Clearly, when feeding based on averages (either live weight or across sexes), the compromise implied will affect nutrient excretion. First, when feeding by average weight whether for one phase or multiple phases, at some point the pig is overfed while at other times the pig is underfed. Obviously, when the pig is overfed the unused nutrients are converted to waste, which represents an environmental burden. However, there is also an environmental impact associated with underfeeding when deficiencies result in slower growth and/or poorer feed efficiency.

Table 4. Effect of phase feeding on nitrogen excreted in pigs from 25 to 105 kg.1

<table>
<thead>
<tr>
<th></th>
<th>Single feed (17% CP)</th>
<th>Two-feedsa (17% then 15% CP)</th>
<th>Three-feedsb 17%, followed by 15%, followed by 13% CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen output, g/day</td>
<td>31.9</td>
<td>29.0</td>
<td>26.7</td>
</tr>
<tr>
<td>Percentage of two-feed strategy</td>
<td>110</td>
<td>100</td>
<td>92</td>
</tr>
</tbody>
</table>

aCrude protein changed at 55 kg
bCrude protein changed at 50 and 75 kg
1from Henry and Dourmad, 1993

It has been demonstrated that improving growth and feed efficiency can reduce the environmental impact of livestock production. As previously mentioned, genetic lines that have higher lean content typically have better feed efficiency compared to lines that have less lean growth capacity. However, there are numerous other factors that can influence the growth and feed efficiency of grow-finish pigs. Certainly, health challenges can reduce growth and feed efficiency and could contribute to increased environmental impact of pork production. Further, diseases that result in poorer absorption of dietary nutrients would have a negative environmental
Reducing the environmental impact of swine production

Maintaining a healthy herd can beneficially affect both the pork producer’s bottom line and the environment. Other environmental and management factors can affect feed efficiency and, hence, alter the environmental burden associated with the farm. Such factors include poor ventilation, overcrowding, mycotoxins in feed, and a host of other factors. Additionally, proper feed processing influences nutrient availability by reducing anti-nutritional factors (Han et al., 2001).

FEED WASTAGE

Another obvious way to reduce the environmental impact of pork production is to control feed wastage. Van Heugten and Van Kempen (1999) indicated that feed wastage ranges from 2 to 20% depending on the country and particular study involved. Producers should pay close attention to feeder adjustment to ensure that wastage is minimized. Other factors, such as feeder design, feeder placement, and maintenance can influence feed wastage.

GENETIC INFLUENCES

Crocker and Robinson (2002) demonstrated that there is a genetic component to nutrient content in swine excreta. Table 5, adapted from Crocker and Robison, shows the genetic differences in various nutrient contents in the excreta of swine. Pigs from the maternal line excreted less phosphorus, calcium, copper, zinc, and iron than did the paternal line or the F1 cross of the two lines. These differences, on a daily basis, may be the result of slower growth and reduced feed intake of the maternal line. However, one should examine the total amount of nutrient excreted over the entire growing phase. Some lines grow faster and excrete more nutrients per day, but excrete less total nutrients throughout the growing phase when compared to lines that grow slower, excrete fewer nutrients on a daily basis, but require a greater number of days to reach the same weight. The Crocker and Robison (2002) study also showed that gilts excrete lower levels of all nutrients compared to barrows.

PROTEIN

Nutritional strategies exist to reduce odor and its components. The obvious method to reduce ammonia emissions would be to reduce the amount of nitrogen excreted (Powers, 2002). This can be accomplished by not over-fortifying crude protein. As previously mentioned, it is possible to meet the amino acid needs of the pig more closely by using crystalline amino acids without over-fortifying the diet with protein sources such as soybean meal. This will allow the diet to more closely meet the pig’s amino acid needs rather than over-fortifying the diet with some amino acids such that other amino acid requirements are met. Excretion of excess amino acids requires energy, which could otherwise be used for growth. For each 1% decrease in dietary crude protein, on average a corresponding ammonia reduction of 10% is observed (Sutton et al., 1997; Kay and Lee, 1997; Blair et al., 1995; Jacob et al., 1994; Aarnink et al., 1993). However, the diets using several crystalline amino acids have tended to be more costly than those diets using soybean meal and thus producers resist implementation.

While reducing dietary crude protein lowers ammonia emissions, it may not help reduce the offensiveness of swine manure odor. A study by Otto et al. (2003) demonstrated that an ammonia emission reduction was obtained when dietary crude protein was reduced from 15% to 9% or 6% in diets that were supplemented with crystalline amino acids. This study also reported that odors from manure from pigs fed these diets were more offensive than the manure from pigs fed the control 15% crude protein diet.

FIBER AND CARBOHYDRATES

Sutton et al. (1998) reported that adding low levels

<table>
<thead>
<tr>
<th>Nutrient, g</th>
<th>Paternal x Paternal line</th>
<th>Paternal line</th>
<th>Maternal line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>962 ± 39†</td>
<td>971 ± 36†</td>
<td>860 ± 43†</td>
</tr>
<tr>
<td>Ammonia</td>
<td>400 ± 28†</td>
<td>442 ± 26†</td>
<td>428 ± 31†</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>291 ± 8†</td>
<td>281 ± 8†</td>
<td>218 ± 9†</td>
</tr>
<tr>
<td>Calcium</td>
<td>281 ± 12†</td>
<td>245 ± 11†</td>
<td>184 ± 13†</td>
</tr>
<tr>
<td>Zinc</td>
<td>8.32 ± 0.33†</td>
<td>9.03 ± 0.30†</td>
<td>5.97 ± 0.37†</td>
</tr>
<tr>
<td>Iron</td>
<td>8.93 ± 0.38†</td>
<td>9.20 ± 0.35†</td>
<td>7.27 ± 0.43†</td>
</tr>
<tr>
<td>Copper</td>
<td>0.94 ± 0.03‡</td>
<td>0.97 ± 0.03‡</td>
<td>0.67 ± 0.04‡</td>
</tr>
<tr>
<td>Potassium</td>
<td>408 ± 17†</td>
<td>418 ± 15†</td>
<td>370 ± 19†</td>
</tr>
</tbody>
</table>

†Adjusted for total weight of pigs (n=8) in pen and feed disappearance. Total output of liquid and solid material for 3 days.
‡, § Values with different superscripts in the same row differ significantly (P<0.01).
1Adapted from Crocker and Robison (2002).
of an oligosaccharide and cellulose reduced the nitrogen content of fresh swine manure, especially the ammonia nitrogen fraction. This study indicated that inclusion of these products stimulates fermentation in the colon, reducing the amount of ammonia in the fresh manure. An additional benefit from the addition of cellulose to the pig’s diet was that the pH of fresh and stored manure was reduced, which would help control ammonia volatilization. This study did not evaluate the economics of the diets or cost of gain for the pigs on test. Other tests have used non-starch polysaccharides and found that total nitrogen excretion did not differ but route of excretion shifted from urine to fecal-excreted nitrogen (Canh et al., 1997). Fecal-excreted nitrogen is less volatile than urine nitrogen.

SULFUR

Many of the odors found most offensive have the ‘rotten egg’ smell indicative of sulfur containing compounds. Shurson et al. (1998) concluded that reducing the amount of dietary sulfur resulted in a 2 to 40% reduction in odor concentrations. One might also theorize that allowing pigs to consume water with high sulfur content might also contribute to manure odor unless other dietary sources of sulfur (i.e. trace mineral mixes) are adjusted accordingly. Reduced sulfur compounds are formed under anaerobic conditions (Mackie et al., 1998). This type of condition is typically found when manure is stored in deep pits or lagoons and is released when the storage structure is agitated. However, other storage systems, like shallow pit recharge and flush, are not likely to facilitate the production of hydrogen sulfide containing compounds within the building. Van Kempen and van Heugten (2003) hypothesized that when these compounds are formed in the anaerobic portion of lagoons, the hydrogen sulfide compounds are metabolized by aerobes in the upper layer of a properly function lagoon. Therefore, strategies are site-specific in nature if they are to be effective.

Diet and air quality

Air quality concerns associated with livestock production, particularly pork production, are largely focused in two areas. First, and foremost, are the odors associated with pork production; and secondly, health concerns associated with exposure to odor compounds (Powers, 2002). Odors from swine manure are emitted during storage, treatment, transport and disposal (Van Horn and Powers, 2004). While the odor-causing compounds emitted are not at toxic levels downwind of swine facilities, the odors can be offensive to those living around swine operations. The offensiveness of livestock odors can be dependent on several factors that are generally subjective. Not all odors smell exactly the same to each person nor is the degree of offensiveness the same from person to person. The odors associated with swine production are caused by the products of anaerobic decomposition of manure (Van Horn and Powers, 2004). Certainly, diet composition can affect the amount of substrate available for anaerobic decomposition. At the present time, the gases most associated with odor concerns are ammonia and hydrogen sulfide. They also are the most commonly regulated. Methane gas is also a concern, but not from an odor standpoint as it is odorless. Methane gas concerns arise from its contribution to greenhouse gases and global warming (Van Horn and Powers, 2004).

Conclusions

Livestock production is becoming more concentrated in many parts of the world and pork production is no exception. The fact that the same number of pigs is being produced on fewer farms does increase the environmental risk. When the environmental risks are combined with the general public concerns about the environment and a larger number of rural households without livestock or experience with animal production, livestock producers must find ways to reduce the environmental impact of their operations. In many cases federal, state, and local laws have imposed regulations designed to reduce the environmental impact of livestock production and producers are forced to comply. The environmental impact of pork production can be reduced through nutritional methods. Many of the nutritional methods can have the added benefit of reducing costs of production to pork producers.

References


Reducing the environmental impact of swine production
Nucleotides and young animal health: can we enhance intestinal tract development and immune function?

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Introduction

The development of antibiotic resistance in humans has led to a growing interest in antibiotic-free animal production worldwide (Bager et al., 2000). Unconventional management, feeding practices, and additives that act as alternatives to antibiotics have been tested (Turner et al., 2001; Stein, 2002). Research in human nutrition has demonstrated that the inclusion of nucleotides in parenteral formulas and infant milk formulas improves intestinal health and the development of the immune system in infants. Because of their role in maintaining intestinal health, dietary nucleotides may act as an alternative to antibiotics in the feeding of young animals. If this were true it would provide the livestock industry with an alternative when looking to formulate antibiotic-free feeds. This would be helpful in particular in diets for young animals where in-feed antibiotics are most efficient. However, very little data relative to the young animal’s need for nucleotides exist; and only limited information is available about the role of nucleotides in the development of the immune system and intestinal health. The objective of the current contribution is to give an update on the current understanding of the roles and functions of nucleotides in young animal feeding.

Nucleotide biochemistry and nomenclature

Nucleotides are ubiquitous molecules with considerable structural diversity. They are composed of a nitrogenous base linked to a pentose sugar to which at least one phosphate group is attached (Figure 1). The pentose sugar may either be a ribose for a ribonucleic acid (RNA) or a 2’-deoxyribose for a deoxyribonucleic acid (DNA). The nitrogenous base can be either a purine or a pyrimidine. Pyrimidine

Figure 1. Structure of a nucleotide.
bases are composed of six-membered rings and comprise uridine, cytosine, and thymine (Table 1). Purine bases have an additional five-membered ring and comprise adenine, guanine, and hypoxanthine. The phosphate group may be in a mono-, di-, or triphosphate form, and is commonly esterified to the C-5′ hydroxyl group of the pentose sugar (Rudolph, 1994).

When the phosphate group is absent, the compound is known as a nucleoside. Nucleosides are formed by a nitrogenous base attached to a pentose sugar through a glycosidic linkage between the N-1 nitrogen of a pyrimidine or the N-3 nitrogen of a purine, and the C-1 carbon of the pentose sugar (Voet and Voet, 1995). A chain of nucleotides attached together via a phosphodiester linkage at the 3C and 5C positions of neighboring ribose units are called polynucleotides or nucleic acids. Nucleic acids conjugated to proteins are called nucleoproteins.

Table 1. Nucleotide nomenclature.

<table>
<thead>
<tr>
<th>Base: Product</th>
<th>Nucleoside</th>
<th>Ribo-nucleotidea</th>
<th>Deoxyribo-nucleotideb</th>
<th>Diphosphate nucleotidec</th>
<th>Triphosphate nucleotided</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purines:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenine</td>
<td>Adenosine</td>
<td>AMP</td>
<td>dAMP</td>
<td>ADP/ dADP</td>
<td>ATP/ dATP</td>
</tr>
<tr>
<td>Guanine</td>
<td>Guanosine</td>
<td>GMP</td>
<td>dGMP</td>
<td>GDP/ dGDP</td>
<td>GTP/ dGTP</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>Inosine</td>
<td>IMP</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pyrimidines:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytosine</td>
<td>Cytidine</td>
<td>CMP</td>
<td>dCMP</td>
<td>CDP/ dCDP</td>
<td>CTP/ dCTP</td>
</tr>
<tr>
<td>Thymine</td>
<td>Thymidine</td>
<td>UMP</td>
<td>dUMP</td>
<td>UDP/ dUDP</td>
<td>UTP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dTMP</td>
<td>dTDP</td>
<td>dTTP</td>
<td></td>
</tr>
</tbody>
</table>
|              | AMP = adenosine 5′-monophosphate; GMP = guanosine 5′-monophosphate; IMP = inosine 5′-monophosphate; CMP = cytidine 5′-monophosphate; UMP = uridine 5′-monophosphate
|              | dAMP = deoxyadenosine 5′-monophosphate; dGMP = deoxyguanosine 5′-monophosphate; dIMP = deoxyinosine 5′-monophosphate; dCMP = deoxycytidine 5′-monophosphate; dUMP = deoxyuridine 5′-monophosphate; dTMP = deoxythymidine 5′-monophosphate
|              | ADP = adenosine 5′-diphosphate; dADP = deoxyadenosine 5′-diphosphate; GDP = guanosine 5′-diphosphate; dGDP = deoxyguanosine 5′-diphosphate; dCDP = deoxycytidine 5′-diphosphate; dCDP = deoxythymidine 5′-diphosphate; UDP = uridine 5′-diphosphate; dUDP = deoxyuridine 5′-diphosphate; dTDP = deoxythymidine 5′-diphosphate
|              | ATP = adenosine 5′-triphosphate; dATP = deoxyadenosine 5′-triphosphate; GTP = guanosine 5′-triphosphate; dGTP = deoxyguanosine 5′-triphosphate; CTP = cytidine 5′-triphosphate; dCTP = deoxycytidine 5′-triphosphate; UTP = uridine 5′-triphosphate; dTTP = deoxythymidine 5′-triphosphate

Table 2. Nucleotide concentration in some commonly used feed ingredients (as is basis).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>5′CMP</th>
<th>5′AMP</th>
<th>5′GMP</th>
<th>5′UMP</th>
<th>5′IMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>0.002</td>
<td>0.001</td>
<td>0.001</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td>Casein</td>
<td>0.001</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Corn</td>
<td>0.003</td>
<td>0.002</td>
<td>0.003</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td>Fish meal</td>
<td>0.026</td>
<td>0.011</td>
<td>0.002</td>
<td>0.001</td>
<td>0.035</td>
</tr>
<tr>
<td>Naked oats</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Plasma protein, spray dried</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td>Red blood cells, spray dried</td>
<td>0.000</td>
<td>0.044</td>
<td>0.003</td>
<td>0.002</td>
<td>0.006</td>
</tr>
<tr>
<td>Soy protein concentrate</td>
<td>0.000</td>
<td>0.001</td>
<td>0.002</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td>Soybean meal, 44%</td>
<td>0.016</td>
<td>0.008</td>
<td>0.003</td>
<td>0.009</td>
<td>0.002</td>
</tr>
<tr>
<td>Whey, dried</td>
<td>0.270</td>
<td>0.019</td>
<td>0.000</td>
<td>0.001</td>
<td>0.004</td>
</tr>
</tbody>
</table>

aData from Mateo et al. (2004a)
The nucleotide concentration in the milk of lactating mammals is species-specific and the concentration of most nucleotides changes during the lactation period (Table 3) (Johke, 1963; Gil and Sanchez-Medina, 1981; Gil and Sanchez-Medina, 1982; Mateo et al., 2004). In a recent experiment in our laboratory, we demonstrated that the concentration of 5′AMP, 5′CMP, 5′GMP, 5′IMP, and 5′UMP in porcine milk changed during the initial two weeks of lactation, but was relatively constant after that (Mateo et al., 2004b).

Because of the species differences in milk nucleotide concentration, it is possible that the nucleotide requirement may also vary among species, but at this point there are no data available on the nucleotide requirements of animals. The demand for nucleotides increases during periods of stress and rapid growth. Therefore, the requirement may be elevated during the immediate post-weaning period of livestock species. Current research in our laboratory is addressing this hypothesis.

Digestion, absorption and metabolism of nucleotides

Dietary nucleoproteins, nucleic acids, and nucleotides need to be enzymatically hydrolyzed prior to absorption because only nucleosides, bases, and small amounts of nucleotides are absorbed. This process takes place in the small intestine. Endonucleases, phosphodiesterases, and nucleoside phosphorylase are the major enzymes involved in this process (Figure 2). These enzymes originate from the brush border epithelium (Markiewicz, 1983; Morley et al., 1987), pancreatic juice (Weickman et al., 1981), and bile (Holdsworth and Coleman, 1975). In contrast to adults, the exact metabolism of nucleic acids ingested by infants is unknown (Carver and Walker, 1995). However, an attempt to evaluate the capability of infants to metabolize nucleic acids and nucleotides was made by Thorell et al. (1996), who suggested that enzymes for nucleotide catabolism are present

### Table 3. Concentration of adenosine 5′-monophosphate (AMP), guanosine 5′-monophosphate (GMP), inosine 5′-monophosphate (IMP), citidine 5′-monophosphate (CMP), and uridine 5′-monophosphate (UMP) in milk from different species (µmoles/100ml)ab.

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Milk</th>
<th>5-7</th>
<th>8</th>
<th>10-11</th>
<th>14-15</th>
<th>21</th>
<th>28-31</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP</td>
<td>Human</td>
<td>2.24e</td>
<td>-</td>
<td>-</td>
<td>2.60e</td>
<td>-</td>
<td>2.02e</td>
</tr>
<tr>
<td></td>
<td>Bovine</td>
<td>3.15f</td>
<td>1.80c</td>
<td>-</td>
<td>2.91f</td>
<td>1.81d</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Caprine</td>
<td>11.09a</td>
<td>6.30e</td>
<td>12.20f</td>
<td>2.79e</td>
<td>-</td>
<td>4.07d</td>
</tr>
<tr>
<td></td>
<td>Ovine</td>
<td>-</td>
<td>15.67d</td>
<td>-</td>
<td>11.87d</td>
<td>-</td>
<td>8.47d</td>
</tr>
<tr>
<td></td>
<td>Equine</td>
<td>-</td>
<td>-</td>
<td>0.50e</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Porcine</td>
<td>12.80f</td>
<td>-</td>
<td>-</td>
<td>6.80f</td>
<td>4.30f</td>
<td>3.00f</td>
</tr>
<tr>
<td>CMP</td>
<td>Human</td>
<td>3.10f</td>
<td>-</td>
<td>-</td>
<td>2.64e</td>
<td>-</td>
<td>1.87f</td>
</tr>
<tr>
<td></td>
<td>Bovine</td>
<td>3.02f</td>
<td>6.20c</td>
<td>-</td>
<td>4.90f</td>
<td>4.12d</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Caprine</td>
<td>8.07d</td>
<td>5.86c</td>
<td>-</td>
<td>2.28e</td>
<td>-</td>
<td>3.55d</td>
</tr>
<tr>
<td></td>
<td>Ovine</td>
<td>-</td>
<td>23.30f</td>
<td>-</td>
<td>7.17d</td>
<td>-</td>
<td>8.70f</td>
</tr>
<tr>
<td></td>
<td>Equine</td>
<td>-</td>
<td>-</td>
<td>1.50e</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Porcine</td>
<td>7.10f</td>
<td>-</td>
<td>-</td>
<td>3.50f</td>
<td>2.30f</td>
<td>2.50f</td>
</tr>
<tr>
<td>GMP</td>
<td>Human</td>
<td>0.50e</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.32f</td>
</tr>
<tr>
<td></td>
<td>Bovine</td>
<td>0.83d</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Caprine</td>
<td>-</td>
<td>-</td>
<td>1.70c</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Ovine</td>
<td>-</td>
<td>1.50d</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Equine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Porcine</td>
<td>14.00f</td>
<td>-</td>
<td>-</td>
<td>10.20f</td>
<td>6.00f</td>
<td>7.10f</td>
</tr>
<tr>
<td>IMP</td>
<td>Porcine</td>
<td>2.60f</td>
<td>-</td>
<td>-</td>
<td>1.40f</td>
<td>0.90f</td>
<td>0.40f</td>
</tr>
<tr>
<td>UMP</td>
<td>Bovine</td>
<td>2.87d</td>
<td>-</td>
<td>-</td>
<td>1.30d</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Caprine</td>
<td>12.37d</td>
<td>12.59d</td>
<td>5.90f</td>
<td>16.08d</td>
<td>-</td>
<td>12.64d</td>
</tr>
<tr>
<td></td>
<td>Ovine</td>
<td>-</td>
<td>65.16d</td>
<td>-</td>
<td>20.07d</td>
<td>-</td>
<td>26.08d</td>
</tr>
<tr>
<td></td>
<td>Equine</td>
<td>-</td>
<td>-</td>
<td>7.70f</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Porcine</td>
<td>263.10f</td>
<td>-</td>
<td>-</td>
<td>144.00f</td>
<td>122.80f</td>
<td>104.00f</td>
</tr>
</tbody>
</table>

---

ab Number of samples analyzed varied between 4 and 12
b Nucleotide analysis via enzymatic analysis or HPLC.
 Data from Johke (1963)
 Data from Gil and Sanchez-Medina (1981)
 Data from Gil and Sanchez-Medina (1982)
 Data from Mateo et al. (2004b)
in the fetal small intestine and act on the nucleotide containing substrates.

The duodenum has the greatest absorptive capacity (Bronk and Hastewell, 1987). Differences in the efficiency of uptake among nucleosides have been reported with guanosine being taken up most rapidly (Sanderson and He, 1994). Under physiological conditions, nucleotides have a limited capacity to pass through cell membranes (Sanderson and He, 1994). This may be due to the absence of a nucleotide transport system. Nucleotides also have a high negatively charged phosphate group that hinders absorption. Therefore, the nucleoside form is the major vehicle for entry of purines and pyrimidines into the epithelial cells. More than 90% of dietary and endogenous nucleosides and bases are absorbed into the enterocyte (Salati et al., 1984; Uauy, 1989).

From the enterocyte, partial metabolic products of dietary and endogenous nucleosides and nucleosides enter the hepatic portal vein. These molecules are carried to the hepatocytes for further metabolism. From the liver, partial metabolic products of dietary and endogenous nucleotides and nucleosides are released into systemic circulation and enter muscle tissue. If these products are not re-utilized for nucleotide production or are unabsorbed, the purine and pyrimidine bases are catabolized into uric acid and β-alanine or β-aminoisobutyrate (Rudolph, 1994; Carver and Walker, 1995; Thorell et al., 1996). In mammals except for primates, uric acid is further catabolized into allantoin via the enzyme uricase. Allantoin is then excreted into the urine. In avian species and primates, uric acid is excreted via the urine. The catabolic products of pyrimidine bases (i.e., β-alanine and β-aminoisobutyrate) are further metabolized into ammonia, carbon dioxide and acetyl CoA.

**Synthesis of nucleotides**

Humans and animals can synthesize nucleotides *de novo* provided that the precursors are available. This process takes place in the cytosol of hepatocytes where all the enzymes for purine and pyrimidine synthesis are available. The purine IMP is synthesized from α-D-ribose-5-phosphate via a process involving 11 reactions. In the first reaction, α-D-ribose-5-phosphate is phosphorylated at C1 by a phosphate group donated by ATP to form 5-phosphoribosyl-1-pyrophosphate (PRPP). In the second reaction, an N-glycosidic bond is formed to synthesize 5-
Storage of nucleotides

Nucleotide metabolism is characterized by constant synthesis and catabolism. Tracer studies in animals indicate that 2 to 5% of dietary nucleotides are retained in the small intestine, liver, and skeletal muscle tissue pools (Saviano and Clifford, 1978). Increased tissue retention has been reported in young animals (Kobota, 1969) and during fasting (Saviano and Clifford, 1978; Gross and Saviano, 1991). This may be a manifestation of a physiological requirement. Nucleotide pools are larger in differentiated (i.e., cancerous) cells than in undifferentiated (i.e., nonmalignant) cells (Sanderson and He, 1994). This suggests that undifferentiated cells are more dependent on the dietary supply of nucleotides.

Physiological roles of nucleotides

The concentration of ribonucleotides is relatively constant in all cells, while the concentration of deoxyribonucleotides varies with the stage of the cell cycle (Barness, 1994). Nucleotides are the building blocks for nucleic acids (i.e., DNA and RNA). However, nucleotides also have physiological roles in the body such as being a source of energy (i.e., ATP and GTP), functioning as cofactors in oxidation and reduction reactions (i.e., FAD, NAD+, and NADP+), serving as physiological regulators (i.e., cAMP and cGMP), and carrying activated intermediates (i.e., UDP-glucose, CMP-sialic acid, and CDP-choline) and acyl groups (i.e., CoA).

Rapidly dividing tissues have increased DNA replication and RNA synthesis (Cory, 1992). Because of the role of nucleotides in RNA synthesis, regeneration of new tissue after injury or relative nutrient deficiency is accelerated (Iwasa et al., 1997; Yamauchi et al., 1998). Replication of DNA occurs during the S phase of the cell cycle, and during this time, the enzyme activity is increased for purine and pyrimidine synthesis and nucleotide interconversion (Carver and Walker, 1995). Therefore, there is an increase in demand for nucleotides during cell division and growth (Tsujinaka et al., 1999).

Dietary nucleotide supplementation has been associated with both humoral and cellular immunity, but the exact mechanism has not been elucidated. Nucleotide deprivation caused the arrest of T-cells in the G phase of the cell cycle, preventing a response to various immunological signals that occur by transition to the S phase (Kulkarni et al., 1994). Nucleotide deprivations also caused a decrease in phagocytic activity, lymphokine production, and/or inhibited lymphocyte maturation (Paubert-Braquet et al., 1992). Dietary nucleotides contribute to the circulating pool of nucleosides available to stimulate leukocyte production (Kulkarni et al., 1994; Carver and Walker, 1995). Therefore, there is an elevated need for nucleotides indicated during periods of immunological challenges.

Dietary factors play a role in the antibody response to immunization of infants. Infants fed milk formula fortified with nucleotides had better responses to immunization as evidenced by an increase in humoral antibody response (Fanslow et al., 1988; Pickering et al., 1998) and increased cytokine production (Carver et al., 1991). *In vivo* studies in mice show similar results to nucleotide supplementation (Jyonouchi et al., 1993; Jonouchi, 1994). Nucleotide-free diets supplemented with single
nucleotides (i.e., AMP, GMP, or UMP), have been shown to increase immunoglobulin concentration (Navarro et al., 1996). Dietary supplementation of purified nucleotides to milk replacers fed newborn bull calves challenged with lipopolysaccharide (LPS), resulted in a trend toward higher mean IgG levels in supplemented compared to the unsupplemented calves (Oliver et al., 2003). Nucleotide supplementation also increased lymphocyte stimulation to phytohaemagglutinin and concanavalin-A challenges in weaning piglets by 50 and 30%, respectively (Zomborsky-Kovacs et al., 1998). Similar results were observed to challenges by keyhole limpet hemocyanin (KLH) and non-specific T-cell mitogens in piglets fed yeast RNA (Cameron et al., 2001). Pigs with pathogenic E. coli infection fed diets supplemented with Nupro™, a yeast extract as a source of nucleotides, have reduced diarrhea and improved weight gain and feed conversion compared to pigs fed the control diet (Maribo, 2003). Results of these studies imply that dietary sources of nucleotides play a role in developing, maintaining, and enhancing the immune system.

Dietary nucleotides enhance intestinal absorption of iron, affect lipoprotein and long chain polyunsaturated fatty acid metabolism, have trophic effects on the intestinal mucosa and liver, and reduce the incidence of diarrhea (Cosgrove, 1998; Schlimme et al., 2000). Fecal flora of infants fed a nucleotide-supplemented commercial milk formula had a predominance of bifidobacteria (Tanaka and Mutai, 1980), while enterobacteria predominated in the fecal flora of infants fed a commercial formula without nucleotide supplementation (Uauy, 1994). These studies suggest that nucleotide supplementation may positively influence the microflora in the gastrointestinal tract, which leads to a lowering of gastric pH and hinders the proliferation of pathogenic bacterial species as evidenced by a lower rate of diarrhea (Yu, 1998).

Nucleotide supplementation increased bacterial resistance of mice inoculated with Candida albicans (Fanslow et al., 1988) and Staphylococcus aureus (Kulkarni et al., 1986; Carver, 1994). Intraperitoneal administration of nucleotides and nucleosides decreased bacterial translocation, number of colony forming units, and increased the survival of mice against methicillin-resistant S. aureus (Yamamoto et al., 1997). Therefore, nucleotides are capable of increasing resistance to a wide range of potentially pathogenic bacteria.

Dietary nucleotides from yeast extract supplemented in calf milk replacers improved intestinal health as demonstrated by improved fecal scores compared to the non-supplemented control group (Oliver et al., 2002). In piglets, nucleotides in concentrations similar to those in human milk exert a protective effect in the intestinal lumen against an inflammatory response to ischemia-reperfusion (Bustamante et al., 1994).

Dietary nucleotides enhance the growth and maturation of intestinal epithelial cells as evidenced by an increased formation of mucosal protein, DNA, taller villi in the small intestine and increased maltase:lactase enzyme ratio (Uauy et al., 1990; Carver, 1994). Dietary nucleotides may also stimulate differentiation (Sanderson and He, 1994). Parenteral supplementation of nucleic acids supports mucosal cell proliferation and function as demonstrated by increased mucosal wet weight, protein and DNA contents, villous height, but not crypt depth, and narrower tight junctions of the jejunal width (Kishibuchi et al., 1997; Tsujinaka et al., 1999). The development of the gastrointestinal tract directly affects the degree of nutrient absorption and ultimately, animal growth. Because of the role of nucleotides in maintaining intestinal morphology and maturation, dietary nucleotides may be needed during the immediate post-weaning period to maintain the structure and growth of the intestinal tract.

**Are dietary nucleotides needed in diets for weanling pigs?**

The need for nucleotides is elevated during periods of rapid growth, during periods of stress, and in immuno-compromised animals. In newly weaned pigs, all of these factors are present — therefore, it is expected that they would have a high requirement for nucleotides during this period. Because nucleotide synthesis is an energy and glutamine-requiring process and because newly weaned pigs are often deficient in both energy and glutamine, it is possible that pigs are not able to synthesize sufficient quantities of nucleotides during the immediate post-weaning period. If this is correct, dietary nucleotides must be supplied. In a typical starter diet for weanling pigs, the concentration of 5′CMP is close to the concentration found in sow’s milk during the last half of lactation, but the concentrations of 5′AMP, 5′GMP, 5′IMP, and 5′UMP are much lower than in sow’s milk (Table 4). Assuming that the concentrations of nucleotides in sow’s milk are representative of the
Table 4. Calculated nucleotide concentration of a starter diet for weanling pigs.

<table>
<thead>
<tr>
<th>Nucleotide (ppm)</th>
<th>Total in starter diet</th>
<th>Sows milk</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CMP</td>
<td>AMP</td>
<td>GMP</td>
</tr>
<tr>
<td></td>
<td>58.99</td>
<td>6.46</td>
<td>2.03</td>
</tr>
<tr>
<td></td>
<td>56.00</td>
<td>117.50</td>
<td>185.5</td>
</tr>
<tr>
<td></td>
<td>2.99</td>
<td>-111.04</td>
<td>-183.47</td>
</tr>
</tbody>
</table>

aData from Mateo et al. (2004a)

bDiet formulated to contain the following feed ingredients: Corn, 49.32%; Whey powder, 20%; Soybean meal, 8%; Fish meal, 8%; spray dried protein plasma, 7.5%; vitamins, minerals, oil, and crystalline amino acids, 7.18%.

cData from Mateo et al. (2004b)

requirement of the piglets, it is easily concluded that a starter diet for young pigs is deficient in four of the five nucleotides. It may, therefore, be beneficial to add additional nucleotides to such diets. In a recent experiment in our laboratory, we added nucleosides to a deficient starter diet. We used nucleosides rather than nucleotides because dietary nucleotides need to be digested to nucleosides before absorption. Blood samples and fecal samples were collected from weanling pigs on the day of weaning, on day 7 post-weaning and on day 14 post-weaning. Blood samples were analyzed for IgG concentrations, while the microbial flora was quantified in the samples. Results of the experiment showed that fecal counts of *Clostridium perfringens* were reduced in samples collected from pigs fed a nucleoside-supplemented diet as compared to the non-supplemented control diet. On day 14 post-weaning, the fecal counts of *Lactobacillus acidophilus* and *Bifidobacterium* spp. also were higher in pigs fed the nucleoside-containing diet (Mateo et al., 2004a). These results indicate that nucleoside supplementation during the immediate post-weaning period may positively influence the gastrointestinal microflora by decreasing *Cl. perfringens* and increasing *L. acidophilus* and *Bifidobacterium* spp. This observation was subsequently confirmed in an in vitro experiment that showed that nucleotides have antibacterial properties against *Cl. perfringens* and *E. coli*. The implication of this finding is that pigs fed diets supplemented with nucleosides may have improved intestinal health, less scouring, and improved performance. Although no improvement in serum IgG was observed in the above experiment, the improved intestinal health may also lead to an improvement in the immune system of animals fed diets supplemented with nucleotides. A reduced concentration of enterobacteria and an increased number of probiotic bacteria (i.e. *L. acidophilus* and *Bifidobacterium* spp.) may also result in improved intestinal morphology and improved nutrient uptake. This hypothesis is being tested in a current research project at South Dakota State University.

**Conclusion**

Nucleotides are molecules with considerable structural diversity. They are composed of a nitrogenous base linked to a pentose sugar to which at least one phosphate group is attached. Feed or food ingredients containing cellular elements are potential sources of nucleotides. Nucleotides have many important physiological, gastrointestinal, and immunological functions in the body. The exact metabolism of nucleic acids ingested by young animals is unknown. Synthesizing nucleotides de novo is metabolically costly compared to synthesis via the Salvage Pathway. During periods of rapid growth and development, disease challenges, injury or stress, dietary nucleotide supplementation may be beneficial because of the role of nucleotides in developing and enhancing immunity, maintaining intestinal health, and preserving energy. However, more research is needed to verify this hypothesis.

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Dairy and beef cattle
Evaluating inoculants for forage crops in Argentine beef and milk grazing systems: effects on silage quality and system profitability

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Introduction

Beef and milk production in Argentina rely mainly on grasslands and improved pastures; and conserved forages or concentrates are fed to the animals generally when DM availability or nutrient content of pastures is insufficient to satisfy animal requirements. Among conserved forage alternatives, silage production is increasing because of its many advantages over other supplements including:

- Less dependence on weather compared with hay.
- Nutrient content maintained, similar to field crop.
- Higher DM production at low cost compared with grains.

Due to the high quality of pastures and relatively low silage inclusion in Argentine diets (<30%, DM basis), good animal performance was obtained when silages supplemented pastures compared with ad libitum grazing, as shown by Abdelhadi et al. (2001; 2003) and Abdelhadi and Santini (2003). An increased stocking rate is the only benefit of corn or grain sorghum silage supplementation of pasture.

Nevertheless, Argentina’s producers are far from having the high quality silages needed in order to justify increasing silage use in rations or to replace concentrate in diets fed high producing animals. If we analyze corn silage, one of the easier crops for making good silage due to its high water soluble carbohydrate (WSC): buffering capacity (BC) ratio, it is clear that digestibility coefficients are lower than in other countries, from a crop of about 75% in vitro dry matter digestibility (IVDMD) at harvest stage (Figure 1).

There are three major events for making good silage: 1) exclusion of air before sealing the silo (generally depends on contract workers); 2) rapid rate and extent of pH drop (depends on the extent of fermentation); and 3) storage phase (depends on farmer management) (Kung, 2001).

Figure 1. Corn silage in vitro DM digestibility (IVDMD) around the world (Data from: Schroeder et al., 2000; INRA, 1989; Roth and Undersander, 1995; Deinum and Struik, 1985; De Boever et al., 1997).
The data in Figure 1 come from samples taken in the silo, not in the feedbunk, and so are the result of Events 1 and 2. Because the work of contractors is often difficult for the farmer to manage, our research was directed at controlling the fermentation process. Together with representatives from Alltech Argentina and Monsanto, we conducted a series of studies to evaluate utilization of Sil-All™ inoculant to increase quality of ensiled crops and hence animal production and profitability of pasture based systems at the El Encuentro farm (35°23´S; 58°25´W).

Effects of Sil-All™ inoculation on fermentation and nutritive value of grain sorghum (whole head) ensiled at two stages of maturity

METHODS

The same grain sorghum hybrid was seeded on December 3 and 20, 2002 on a commercial dairy farm (El Recuerdo). Harvest occurred on March 26, 2003, therefore we had the same cultivar at two stages of maturity (milk and dough stages). A Class Jaguar 860 harvester equipped with a grain cracker was used; and the chop length was 7.9 mm. Within 30 min of chopping, samples of fresh material from each stage of maturity were frozen and triplicate mini silos constructed of PVC pipe (10 x 39 cm) were made with or without Sil-All™ inoculation. Weights of silos both empty and full were recorded, and silos were then stored at ambient temperature of about 15 to 20ºC. After a three month storage period (from March to June, 2003) final weights of the full silos were recorded and the silage was hand-mixed thoroughly. Dry matter (DM) recovery was calculated as in Kung et al. (2003) and pack density in each mini silo was estimated as in Johnson et al. (2002). Approximately 50% of the mixed material was frozen and the remainder was exposed to air for three days after which samples were frozen. Frozen samples of each material pre-ensiling (PRE), post-ensiling (POS) and post-air exposure (PAE), were oven-dried (60ºC), ground through a 1 mm screen (Willey Mill, Philadelphia, PA) and analyzed for DM, in vitro organic matter (OM) degradability (Villalba, 2001), WSC (Bailey, 1958), crude protein (CP) (Horneck and Miller, 1998) and NDF (van Soest, 1991); plus pH (Cole Parmer portable pH meter) and ammonia-N (NH₃-N) (Weatherburn, 1967) in POS and PAE samples. Statistical analysis was conducted using the general linear model in SAS (1989) using a completely randomized design with a 2 x 2 factorial arrangement (two stages of maturity with or without Sil-All™ inoculation).

RESULTS

DM recovery and fermentation

A maturity x inoculant interaction was found (P<0.06) for DM recovery. Inoculation increased (P<0.08) silage DM recovery from 92.4% (control) to 97.9% in silages harvested at the milk stage of maturity, while no effect was noted (P<0.25) in silage harvested at the dough stage.

Parameters associated with silage fermentation are presented in Table 1. Sil-All™ inoculation decreased silage temperature after three days of air exposure (P<0.01) by 0.7 and 1.4 ºC for milk and late dough grain stages of maturity, respectively. No effects of inoculation were detected for pH (P<0.93) or NH₃-N (P<0.53), while air exposure increased pH and decreased NH₃-N of silages at either stage of maturity (P<0.01).

Table 1. Sil-All™ effects on temperature and silage fermentation of grain sorghum (whole head) at milk or late dough stages of maturity†.

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Temperature</th>
<th>Fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ºC)</td>
<td>pH</td>
</tr>
<tr>
<td></td>
<td>Post air exposure</td>
<td>Post-ensiling exposure</td>
</tr>
<tr>
<td>Milk stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>19.0 (0.0)</td>
<td>4.13 (0.0)</td>
</tr>
<tr>
<td></td>
<td>18.3 (0.6)</td>
<td>4.17 (0.0)</td>
</tr>
<tr>
<td>Sil-All™</td>
<td>18.3 (0.6)</td>
<td>4.17 (0.0)</td>
</tr>
<tr>
<td>Late dough stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20.0 (0.0)</td>
<td>4.71 (0.1)</td>
</tr>
<tr>
<td></td>
<td>18.6 (0.6)</td>
<td>4.82 (0.1)</td>
</tr>
<tr>
<td>Sil-All™</td>
<td>18.6 (0.6)</td>
<td>4.82 (0.1)</td>
</tr>
<tr>
<td>Maturity effect (M)</td>
<td>0.02 (n.d.)</td>
<td>0.63 (n.d.)</td>
</tr>
<tr>
<td>Sil-All™ effect (S)</td>
<td>0.01 (n.d.)</td>
<td>0.93 (n.d.)</td>
</tr>
<tr>
<td>Sampling time effect (ST)</td>
<td>n.d. (n.d.)</td>
<td>0.01 (n.d.)</td>
</tr>
<tr>
<td>M x S interaction</td>
<td>0.19 (0.19)</td>
<td>0.47 (0.47)</td>
</tr>
<tr>
<td>M x S x ST interaction</td>
<td>n.d. (n.d.)</td>
<td>0.38 (n.d.)</td>
</tr>
</tbody>
</table>

†Treatment means (± standard deviation)

Nutrient content and in vitro degradability

Neither DM, CP nor NDF concentration were affected by inoculation, while WSC in silage increased with Sil-All™ (P<0.05) (Table 2).
Significant maturity effects were detected for nutrient concentrations in pre-ensiled samples ($P<0.01$), which underscores the importance of defining harvest time for grain sorghum silage.

*In vitro* OM degradability was determined after incubation of PRE, POS and PAE samples with ruminal fluid (Table 3). No significant effects of Sil-All$^\text{TM}$ inoculation were noted across the entire incubation time ($P<0.5$), but depending on time of incubation, increases in degradability from 3 to 5 points were noted and should be considered from an economic point of view. Again, increased maturity negatively influenced quality of material at ensiling.

**Effects of Sil-All$^\text{TM}$ inoculation on fermentation and nutritive value of whole-plant grain sorghum silage**

**METHODS**

Grain sorghum (MS2, Morgan hybrid) was direct seeded on December 5, 2002 at the Los Aromos dairy

| Table 2. Sil-All$^\text{TM}$ effects on nutrient concentration of whole-head grain sorghum silage at milk or late dough stages of maturity$^{1,2,3}$.
<table>
<thead>
<tr>
<th>Sample time</th>
<th>DM, %</th>
<th>WSC</th>
<th>CP</th>
<th>NDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling time</td>
<td>PRE</td>
<td>POS</td>
<td>PAE</td>
<td>PRE</td>
</tr>
<tr>
<td>Milk stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>35.1 (0.2)</td>
<td>32.7 (0.2)</td>
<td>32.6 (0.3)</td>
<td>10.9 (2.1)</td>
</tr>
<tr>
<td>Sil-All$^\text{TM}$</td>
<td>35.1 (0.2)</td>
<td>35.3 (1.9)</td>
<td>32.8 (0.6)</td>
<td>10.9 (2.1)</td>
</tr>
<tr>
<td>Late dough stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>42.9 (2.1)</td>
<td>40.2 (0.8)</td>
<td>41.0 (0.6)</td>
<td>8.9 (0.3)</td>
</tr>
<tr>
<td>Sil-All$^\text{TM}$</td>
<td>42.9 (2.1)</td>
<td>38.8 (0.1)</td>
<td>41.8 (2.8)</td>
<td>8.9 (0.3)</td>
</tr>
</tbody>
</table>

1Treatment means (± standard deviation); 2PRE= pre-ensiling, POS= post-ensiling, PAE= after 3 days air exposure

3DM= dry matter, WSC= water soluble carbohydrates, CP= crude protein, NDF= neutral detergent fiber

| Table 3. Sil-All$^\text{TM}$ effects on *in vitro* organic matter degradability of whole-head grain sorghum silage at milk or late dough stages of maturity$^{1,2,3}$.
<table>
<thead>
<tr>
<th>Hours of incubation</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling time</td>
<td>PRE</td>
<td>POS</td>
<td>PAE</td>
</tr>
<tr>
<td>Milk stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>43.7 (2.0)</td>
<td>38.5 (1.9)</td>
<td>30.6 (2.3)</td>
</tr>
<tr>
<td>Sil-All$^\text{TM}$</td>
<td>43.7 (2.0)</td>
<td>36.5 (3.8)</td>
<td>27.0 (1.4)</td>
</tr>
<tr>
<td>Late dough stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>28.0 (1.5)</td>
<td>31.4 (1.1)</td>
<td>27.4 (2.2)</td>
</tr>
<tr>
<td>Sil-All$^\text{TM}$</td>
<td>28.0 (1.5)</td>
<td>31.4 (1.7)</td>
<td>30.1 (2.8)</td>
</tr>
</tbody>
</table>

1Treatment means (± standard deviation); 2PRE= pre-ensiling, POS= post-ensiling, PAE= after 3 days air exposure
farm (35°10’S; 58°14’W). Harvesting occurred on March 27, 2003 when grain was at the milk stage. Ensiling, sampling methods and quality determinations were as in the first experiment. Data were analyzed as a completely randomized design and treatment comparisons were obtained by ANOVA using the PROC GLM procedures of SAS (1988).

RESULTS

**DM recovery and fermentation**

Dry matter recovery was not statistically different, however the numerical increase from 92.1% (control) to 95.1% with Sil-All™ inoculation could be of economic relevance. Temperature after three days exposure to air was unaffected (P<0.34) by inoculation, as were pH and NH₃-N values (Table 4).

**Nutrient content and in vitro degradability**

There were no significant effects of inoculation on nutrient quality of silage samples taken either after fermentation or following aerobic exposure (Table 5). *In vitro* OM degradability of air-exposed silage was lower than the pre-ensiled samples (P<0.01) (Table 6). Depending on time of incubation, Sil-All™ tended to reduce losses in degradability (by 3.5 points).

| Table 4. Sil-All™ effects on temperature and silage fermentation of whole-plant grain sorghum silage at the milk stage of maturity1,2. |
|---|---|
| Silage Fermentation | |
| Temperature (ºC) | pH | NH₃-N | |
| Sampling time | PAE | POS | PAE | POS | PAE | POS | PAE | |
| Control | 22.3 | 4.1 | 5.4 | 6.9 | 3.5 |
| (2.8) | (0.1) | (1.3) | (1.2) | (1.9) | |
| Sil-All™ | 23.6 | 4.1 | 4.8 | 6.7 | 2.8 |
| (0.5) | (0.0) | (0.3) | (0.6) | (1.3) | |
| Sil-All™ effect (S) | 0.34 | 0.52 | 0.61 |
| Sampling time effect (ST) | n.d. | 0.04 | 0.01 |
| S x ST interaction | n.d. | 0.42 | 0.76 |

1Treatment means (± standard deviation).

2POS= post-ensiling, PAE= after 3 days air exposure

| Table 5. Sil-All™ effects nutrient concentration of whole-plant grain sorghum silage at milk stage of maturity1,2,3. |
|---|---|
| Nutrient content (% of DM) | |
| WSC | CP | NDF | |
| Sampling time | PRE | POS | PAE | PRE | POS | PAE | PRE | POS | PAE | PRE | POS | PAE | |
| Control | 29.4 | 27.5 | 27.1 | 10.1 | 6.9 | 7.7 | 6.6 | 7.1 | 7.6 | 48.2 | 52.2 | 54.4 |
| (0.6) | (0.3) | (0.5) | (0.6) | (0.3) | (1.3) | (0.6) | (0.5) | (0.2) | (1.7) | (0.7) | (2.4) |
| Sil-All™ | 29.4 | 28.6 | 27.4 | 10.1 | 6.9 | 8.0 | 6.6 | 7.6 | 8.0 | 48.2 | 50.8 | 52.0 |
| (0.6) | (1.9) | (0.3) | (0.6) | (0.7) | (1.4) | (0.6) | (0.7) | (1.7) | (1.6) | (1.9) | |
| Sil-All™ effect (S) | 0.31 | 0.80 | 0.29 | 0.25 |
| Sampling time effect (ST) | 0.01 | 0.01 | 0.01 | 0.01 |
| S x ST interaction | 0.57 | 0.89 | 0.74 | 0.69 |

1Treatment means (± standard deviation).

2PRE= pre-ensiling, POS= post-ensiling, PAE= after 3 days air exposure

3DM= dry matter, WSC= water soluble carbohydrates, CP= crude protein, NDF= neutral detergent fiber

| Table 6. Sil-All™ effects on *in vitro* organic matter degradability of whole-plant grain sorghum silage at milk stage of maturity1,2. |
|---|---|
| In *in vitro* organic matter degradability (%) | |
| Hours of incubation | 24 | 48 | 72 | |
| Sampling time | PRE | POS | PAE | PRE | POS | PAE | PRE | POS | PAE | |
| Control | 43.3 | 36.2 | 33.2 | 64.1 | 55.8 | 51.1 | 73.6 | 65.2 | 60.9 |
| (2.8) | (4.4) | (2.3) | (2.2) | (6.2) | (5.1) | (2.0) | (7.3) | (5.6) | |
| Sil-All™ | 43.3 | 40.2 | 34.3 | 64.1 | 59.2 | 53.7 | 73.6 | 68.5 | 63.3 |
| (2.8) | (4.5) | (2.2) | (2.2) | (2.4) | (2.8) | (2.0) | (2.7) | (2.5) | |
| Sil-All™ effect (S) | 0.30 | 0.29 | 0.36 |
| Sampling time effect (ST) | 0.01 | 0.01 | 0.01 |
| S x ST interaction | 0.57 | 0.73 | 0.78 |

1Treatment means (± standard deviation)

2PRE= pre-ensiling, POS= post-ensiling, PAE= after 3 days air exposure
Effects of Sil-All™ inoculation on fermentation and nutritive value of whole-plant corn silage

METHODS

Corn (M369, Morgan hybrid) was seeded on October 25, 2002 in Los Aromos dairy farm (35º10´S; 58º14´W). Ensiling, sampling methods and quality determinations were as in the previous experiments, but harvest occurred at the late dough stage of maturity on March 27, 2003. Statistical analysis was as in the second experiment.

RESULTS

DM recovery and fermentation

Inoculation with Sil-All™ increased DM recovery ($P<0.05$) from 94.5% (control) to 99.6%. The added 5.1 percentage points represents a large amount of forage. Sil-All™ inoculation decreased pH ($P<0.05$) measured in samples taken either after silage fermentation or after aerobic exposure (Table 7). Heating was also reduced in the inoculated silage by 1.6°C. Ammonia-N tended to be lower in inoculated silage but was not statistically different.

Table 7. Sil-All™ effects on temperature and silage fermentation of whole-plant corn silage at late dough stage of maturity1,2.

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Silage Temperature (°C)</th>
<th>pH</th>
<th>NH₃-N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PAE</td>
<td>POS</td>
<td>PAE</td>
</tr>
<tr>
<td>Control</td>
<td>21.6</td>
<td>4.34</td>
<td>6.32</td>
</tr>
<tr>
<td>(0.5)</td>
<td>(0.2)</td>
<td>(1.7)</td>
<td>(0.4)</td>
</tr>
<tr>
<td>Sil-All™</td>
<td>20.0</td>
<td>4.13</td>
<td>5.61</td>
</tr>
<tr>
<td>(0.0)</td>
<td>(0.1)</td>
<td>(1.4)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>Sil-All™ effect (S)</td>
<td>0.01</td>
<td>0.05</td>
<td>0.50</td>
</tr>
<tr>
<td>Sampling time effect (ST)</td>
<td>n.d.</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>S x ST interaction</td>
<td>n.d.</td>
<td>0.52</td>
<td>0.71</td>
</tr>
</tbody>
</table>

1Treatment means (± standard deviation).

Nutrient content and in vitro degradability

A significant increase in DM percentage ($P<0.02$) and decreased NDF content ($P<0.09$) were detected in inoculated corn silage (Table 8). Neither WSC nor CP concentrations were affected by inoculation. A trend toward increased in vitro OM degradability was detected after 48 ($P<0.19$) and 72 hrs ($P<0.17$) of incubation in Sil-All™-treated material (Table 9). The added 3.5%
digestible material is similar to values reported for grain sorghum silage.

**Economic evaluation of Sil-All™ inoculation for Argentine systems**

Because silage inclusion in beef and dairy cattle diets in Argentina is only moderate (<30%, DM basis) less benefit will be achieved when increasing silage quality than in other feeding systems. The chief benefit of inoculation in the Argentine system is the added DM recovery, which along with improved silage quality, has the potential to increase animal production and hence economic return.

To calculate profitability of a silage inoculation program for Argentina, we considered DM recoveries and increases in degradability obtained. These data were transformed to extra animal live weight gain (LWG) or milk production using the following criteria:

1) 8.8 kg DM of silage = 1 kg LWG/ha and 5.3 kg digestible DM (DDM) = 1 kg LWG/ha in grazing beef cattle (Abdelhadi et al., 2001; 2003; Abdelhadi and Santini, 2003).

2) 1.45 kg DM of silage = 1 kg milk and 0.87 kg DDM = 1 kg milk in grazing dairy cows (Abdelhadi et al., 2001) (using a mean digestibility of about 60% (DM basis) generally obtained in commercial farms) (Table 10).

Finally, the cost of inoculation was deducted from extra return to obtain the benefit:cost ratio. Benefit:cost ratios of 6.5:1 (beef) and 8.8:1 (dairy) USD per hectare of grain sorghum or corn ensiled were obtained (Table 10).

**Table 10. Benefit:cost ratio of Sil-All™ inoculation in ensiled corn or sorghum silages for supplementing grazing beef or dairy cattle.**

<table>
<thead>
<tr>
<th>Item</th>
<th>Beef</th>
<th>Dairy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extra DM recovery¹, kg/ha</td>
<td>800</td>
<td>800</td>
</tr>
<tr>
<td>Extra digestible DM², kg/ha</td>
<td>700</td>
<td>700</td>
</tr>
<tr>
<td>DM silage/kg LWG or milk, kg</td>
<td>8.8</td>
<td>1.45</td>
</tr>
<tr>
<td>DDM silage/kg LWG or milk, kg</td>
<td>5.3</td>
<td>0.87</td>
</tr>
<tr>
<td>Extra LWG or milk potentially obtained, kg</td>
<td>22.3</td>
<td>1356</td>
</tr>
<tr>
<td>Market prices³ per kg of beef or milk, USD</td>
<td>0.69</td>
<td>0.16</td>
</tr>
<tr>
<td>Extra $ obtained per ha of ensiled crop</td>
<td>153.9</td>
<td>216.9</td>
</tr>
<tr>
<td>Cost of inoculation (USD) for silage yield obtained⁴</td>
<td>24.6</td>
<td>24.6</td>
</tr>
<tr>
<td>Benefit:cost ratio</td>
<td>6.2:1</td>
<td>8.8:1</td>
</tr>
</tbody>
</table>

¹ Mean production DM/ha for sorghum and corn = 20 tons, considering DM recovery of 5% extra by inoculation.

² Mean production DDM/ha for sorghum and corn = 12 tons, considering an extra of digestible DM of 3.5 points which is 5.83% extra of DDM/ha.

³ Expressed in USD, considering local money exchange of 2.9 Argentinean pesos = $1.

⁴ Mean of about 60 tonnes of fresh material per hectare.

**Conclusions**

Even though ensiling grain sorghum and maize is easier due to higher WSC:BC ratio, significant losses have been quantified in Argentine silos. These experiments were conducted to evaluate the Sil-All™ inoculant as a management tool to reduce such losses. Data obtained showed increased DM recovery and in vitro OM degradability of ensiled crops when Sil-All™ was used. In addition, a better fermentation pattern and lower NDF content was reported depending on crop evaluated. Since silages in Argentina supplement grazing cattle, we evaluated these increases in terms of quantity and quality in economic terms, finding a benefit:cost ratio of 6.5:1 (beef) or 8.8:1 (dairy) USD per hectare of grain sorghum or corn ensiled. These results will give farmers the technical and economic information needed to implement an inoculation program for pasture-based systems.

**References**


Optigen® 1200: controlled release of non-protein nitrogen in the rumen

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2Comsen Dairy Consultation, LLC, Dryden, New York, USA

Introduction

Non-protein nitrogen (NPN) includes any compound that contains nitrogen (N) but which is not present in the polypeptide form of proteins. Inexpensive NPN, especially urea, is very useful in ruminant rations because it is hydrolyzed to ammonia in the rumen and can be incorporated by microbes into amino acids and bacterial proteins, which are utilized subsequently by the host animal. As a result, NPN is used in dairy and beef rations as a less expensive alternative to pre-formed proteins of plant origin such as soybean meal or cottonseed meal, or of animal origin such as fish meal or blood meal.

A variety of factors must be considered when utilizing urea. The amount of urea that can be used is limited because of the rapid hydrolysis of urea to ammonia in the rumen. This rapid breakdown to ammonia can occur at a much faster rate than ammonia assimilation by the rumen bacteria, resulting in accumulation and escape of ammonia from the rumen (Satter and Roffler, 1975). As a result, urea can only be used as a source of nitrogen when there is an adequate supply of readily fermentable carbohydrate available to synthesize bacterial protein. Because ammonia produced in the rumen is used for bacterial growth and bacterial growth is dependent on energy availability, it is important that the rate of ammonia production in the rumen be coordinated with the rate of carbohydrate fermentation. If the rates for ruminal ammonia production and energy digestion are not synchronized, ammonia concentration in the rumen will increase after feeding (Newbold and Rust, 1992; Henning et al., 1993). However, simply increasing supplemental dietary nonstructural carbohydrate (NSC) does not guarantee either an increase in bacterial protein yield or efficiency of bacterial protein synthesis and improved utilization of urea (Stern et al., 1994). In contrast, greater dietary concentrations of NSC have increased the utilization of ruminal ammonia for the synthesis of bacterial protein (Hoover and Stokes, 1991). These findings indicate that microbial metabolism in the rumen is a complex process requiring an understanding of both the rate and extent of carbohydrate digestion and ammonia supply for efficient bacterial growth in the rumen.

Controlled release non-protein nitrogen compounds

Attempts at synchronizing the ruminal production of ammonia with ruminal energy digestion have focused on the development of controlled release urea compounds for more than 30 years. Previously developed controlled release NPN compounds such as isobutyldine monourea (Mathison et al., 1994), biuret (Löest et al., 2001), Starea (Bartley and Deyoe, 1975), formaldehyde-treated urea (Prokop and Klopfenstein, 1977), and linseed oil-coated urea (Forero et al., 1980), have not been as advantageous as urea because a substantial part of the NPN in these compounds may leave the rumen without being converted to ammonia, thus reducing its incorporation into bacterial protein. They are also less advantageous because the ammonia formation from these compounds in the rumen, though slower than urea, is still too fast to improve the utilization of nitrogen by rumen bacteria (Owens and Zinn, 1988; Henning et al., 1993).

These data clearly indicate that a new approach to supplying controlled release NPN is needed. Optigen® 1200 is prilled urea coated with a biodegradable
polymer that has a controlled release property. This material is a highly concentrated nitrogen source that can enhance rumen function by supplying nitrogen to rumen bacteria at a rate that optimizes the conversion of nitrogen into bacterial protein. Optigen® 1200 also increases the nitrogen density of the protein fraction of the diet, enhances the growth of fiber utilizing bacteria, and creates more space for the inclusion of digestible fiber and energy in the ration.

Experiments evaluating the efficacy of Optigen® 1200

IN SITU NITROGEN DISAPPEARANCE

In situ nitrogen disappearance of Optigen 1200® was compared with that of urea and soybean meal. In these studies, samples of Optigen 1200, feed grade urea and soybean meal were placed in polyester bags. Sample size was adjusted so that the sample:bag surface area ratio was similar for all nitrogen sources. The bags were suspended in the rumen of a cannulated animal. There were two bags per time point and three replications. Time points for bag incubation were 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24 and 30 hrs. When the polyester bags were removed from the rumen, they were washed with distilled water until the rinse water was clear. The bags were dried in a forced air oven at 60°C. Samples were composited by time point and then analyzed for nitrogen.

In situ nitrogen disappearance of Optigen® 1200 followed a pattern which was more similar to soybean meal than urea (Figure 1). Nitrogen disappearance of urea followed a linear pattern, while the disappearance of nitrogen from Optigen® 1200 followed a 2nd order polynomial pattern. This indicated that for urea there was one rapid nitrogen disappearance rate, but for Optigen® 1200, there were two different disappearance rates. Optigen® 1200 had an intermediate disappearance rate during the first 16 hrs of rumen fermentation. This was followed by a slower disappearance rate from 16 to 30 hrs. This two phase disappearance pattern was similar to the pattern observed for soybean meal. During the first 3 hrs of fermentation, Optigen® 1200 had a faster rate of disappearance than soybean meal, but had a slower rate of disappearance between 3 and 16 hrs of fermentation. These results clearly demonstrate that Optigen® 1200 is a controlled release source of NPN, since typical NPN compounds like urea have a disappearance rate that is much faster than soybean meal.

BACTERIAL PROTEIN SYNTHESIS AND DISAPPEARANCE OF NUTRIENTS IN CONTINUOUS CULTURE FERMENTORS

Studies with rumen-simulating continuous culture fermentors have been conducted to evaluate the effects
of Optigen® 1200 addition on bacterial protein synthesis and disappearance of nutrients. Continuous culture fermentors were inoculated with 1000 ml of rumen fluid and 50 g of digesta taken from a ruminally-cannulated Holstein. Each fermentor was fed the control diet or Optigen® 1200 diet (0.66% Optigen® 1200 on a DM basis) at a rate of 75 g DM per day in three equal feedings. The fermentors were operated for a 5-day adjustment period, followed by a 3-day sampling period. During the sampling period, effluent was collected daily and a portion was freeze-dried and ground for the analysis of acid detergent fiber (ADF), neutral detergent fiber (NDF), total carbohydrate (CHO) and organic matter (OM).

Optigen® 1200 resulted in more bacterial protein synthesis and faster disappearance of nutrients compared to the control diet (Figure 2). Bacterial protein synthesis was 6% higher for Optigen® 1200. Optigen® 1200 also increased the disappearance of ADF, NDF, total CHO and OM in the amounts of 16.6, 6.8, 4.0 and 8.0%, respectively. Improved nutrient disappearance with Optigen® 1200 was apparently due to an increased number of bacteria in the rumen, which increased digestion of these nutrients. Ruiz et al. (2002) reported higher DM, N and NDF disappearance when urea was added to the diet of lactating dairy cows. However, Owens and Zinn (1988) reported that while controlled release NPN compounds, such as Starea, biuret, certain coating materials and most complexes of urea with formaldehyde or molasses did help avoid ammonia toxicity, they did not affect nutrient disappearance.

LACTATING DAIRY COWS: EXPERIMENT 1

Two hundred and twenty lactating dairy cows on a commercial dairy in western New York were used to compare a control diet with a diet containing Optigen® 1200. Groups were balanced for days in milk, parity, and milk yield at the start of the experiment. Both groups were fed the control diet from May 16 through June 15. During the baseline period, daily milk weights for individual cows were recorded and DHIA Dairy One (Ithaca, NY) took milk samples every 14 days. The treatment period started on June 16 and ended on August 25, during which time individual cow daily milk weights were again recorded and DHIA took milk samples every 14 days. Individual cow milk samples were analyzed for CP, fat and milk urea N.

The ingredient composition of the diets is shown in Table 1. The amount of forage dry matter was similar between treatments, both containing 22 lbs. The diets contained 41.5% forage and 58.5% concentrate. When Optigen® 1200 was added to the diet, the amounts of urea and soybean meal were reduced and carbohydrates (wheat midds and ground corn) were used to replace these protein supplements. Both diets contained 0.44 lbs of buffer per cow as part of the mineral supplement.

![Figure 2](image_url). Bacterial protein synthesis and disappearance of nutrients in rumen-simulating continuous culture fermentors fed a control diet or a diet that contained 0.66% Optigen® 1200 on dry matter basis.
Dietary nitrogen fraction concentrations are in Table 2. The diets differed in the amount of rumen undegradable protein (RUP), starch and rumen degradable protein (RDP). The addition of Optigen® 1200 to the diet resulted in a 5.55% decrease in RUP content, and a 3.06% increase in starch content as well as a 4.12% increase in RDP content of the diet.

Table 1. Ingredient composition of experimental diets fed during Experiment 1 (DM basis).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control (lb DM/day)</th>
<th>Optigen® 1200 (lb DM/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage</td>
<td>15.38</td>
<td>15.37</td>
</tr>
<tr>
<td>Haylage</td>
<td>6.59</td>
<td>6.59</td>
</tr>
<tr>
<td>Whole cottonseed</td>
<td>4.40</td>
<td>4.40</td>
</tr>
<tr>
<td>Ground corn</td>
<td>13.55</td>
<td>14.00</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>3.85</td>
<td>1.65</td>
</tr>
<tr>
<td>Feed grade urea</td>
<td>0.22</td>
<td>0.10</td>
</tr>
<tr>
<td>Optigen® 1200</td>
<td>0</td>
<td>0.50</td>
</tr>
<tr>
<td>Brewer’s grains</td>
<td>0.97</td>
<td>0.88</td>
</tr>
<tr>
<td>Wheat midds</td>
<td>0</td>
<td>1.76</td>
</tr>
<tr>
<td>Soybean hulls</td>
<td>0.97</td>
<td>0.88</td>
</tr>
<tr>
<td>Soy Plus</td>
<td>1.94</td>
<td>1.76</td>
</tr>
<tr>
<td>Agway Hi-Lysine Protein suppl</td>
<td>0.50</td>
<td>0.51</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>0.29</td>
<td>0.26</td>
</tr>
<tr>
<td>Tallow</td>
<td>0.44</td>
<td>0.49</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.93</td>
<td>0.96</td>
</tr>
<tr>
<td>Custom min/vit plus buffer</td>
<td>1.26</td>
<td>1.18</td>
</tr>
<tr>
<td>Total dry matter</td>
<td>53.00</td>
<td>53.00</td>
</tr>
</tbody>
</table>

Table 2. Nutrient composition of diets fed during Experiment 1 (DM basis).†

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Control</th>
<th>Optigen® 1200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
<td>18.20</td>
<td>18.40</td>
</tr>
<tr>
<td>Degradable protein, % DM</td>
<td>11.65</td>
<td>12.15</td>
</tr>
<tr>
<td>Soluble protein, % of CP</td>
<td>29.81</td>
<td>30.47</td>
</tr>
<tr>
<td>Undegradable protein, % of CP</td>
<td>36.10</td>
<td>34.00</td>
</tr>
<tr>
<td>NFC:DIP2</td>
<td>3.24</td>
<td>3.15</td>
</tr>
<tr>
<td>Net energy, mcal/lb</td>
<td>0.78</td>
<td>0.78</td>
</tr>
<tr>
<td>ADF, %</td>
<td>19.64</td>
<td>19.57</td>
</tr>
<tr>
<td>NDF, %</td>
<td>30.39</td>
<td>31.02</td>
</tr>
<tr>
<td>Forage, % of DM</td>
<td>41.50</td>
<td>41.50</td>
</tr>
<tr>
<td>NFC, %</td>
<td>37.69</td>
<td>38.22</td>
</tr>
<tr>
<td>Starch, %</td>
<td>28.11</td>
<td>28.00</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>1.10</td>
<td>1.10</td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>Potassium, %</td>
<td>1.67</td>
<td>1.38</td>
</tr>
<tr>
<td>Magnesium, %</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Sodium, %</td>
<td>0.44</td>
<td>0.50</td>
</tr>
<tr>
<td>Sulfur, %</td>
<td>0.23</td>
<td>0.23</td>
</tr>
</tbody>
</table>

†Estimated values based on forage analysis and diet composition.

An injection of bST was given to both groups on the same day every 14 days. At the start of the experiment, both groups contained the same percentage of cows receiving bST injections. DHIA test day data were analyzed using the Proc Mixed Model procedure of SAS (1999). Fixed effects in the SAS model included treatment, stage of lactation, and treatment by DHIA test day. Cow was considered as a random effect in this model. Only cows that had four or more DHIA test days during the test period were included in the analysis. For the statistical analysis, data from 206 cows were analyzed. Multiple test days per cow were treated as repeated measures.

Optigen® 1200 significantly altered milk yield (Table 3). This treatment supplied more RDP and more rumen degradable starch compared to the control diet. It is likely that Optigen® 1200 stimulated rumen bacterial growth and ruminal digestion of the diet because milk yield increased 8.14 lbs/day in the group given Optigen® 1200. This represents a 9.74% increase in milk yield. One explanation for the increase in milk yield could be the enhanced ruminal starch digestion. Huber and Herrera-Saldana (1994) reported a 9% increase in milk yield when dry rolled sorghum was replaced with steam-flaked sorghum, which increased ruminal starch digestion. Hoover and Stokes (1991) reported that as the RDP of the diet was increased from 10% to 14% of DM, carbohydrate digestion increased from 60 to 70%.

Table 3. Least squares means of milk yield and percentage of milk fat and protein in Experiment 1.

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>SE</th>
<th>Optigen® 1200</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield, lb/d</td>
<td>83.56</td>
<td>2.65</td>
<td>91.70</td>
<td>2.37</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Milk fat, %</td>
<td>3.72</td>
<td>0.08</td>
<td>3.48</td>
<td>0.07</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fat yield, lb/d</td>
<td>3.11</td>
<td>0.09</td>
<td>3.19</td>
<td>0.08</td>
<td>0.48</td>
</tr>
<tr>
<td>Milk protein, %</td>
<td>3.01</td>
<td>0.03</td>
<td>2.95</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Protein yield, lb/day</td>
<td>2.47</td>
<td>0.07</td>
<td>2.71</td>
<td>0.06</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

It is likely that using Optigen® 1200 as a nitrogen source increased rumen efficiency compared to urea and soybean meal. Milk fat and milk protein content were decreased compared to controls. This was expected since milk volume was increased by 9.74%. Yield of milk fat was unaffected. On the Optigen® 1200 diet, milk protein yield was increased by 8.9% even though RUP was reduced by 5.55%. This suggests that when Optigen® 1200 was used as a nitrogen source, ruminal bacterial protein production was increased.

Based upon animal performance, Optigen® 1200 can be used to enhance rumen function by creating space in the diet to optimize carbohydrate and protein digestion rates. In the present experiment, Optigen® 1200 partially replaced soybean meal and urea in the diet. Feeding 0.5 lbs of Optigen® 1200 allowed for a reduction in soybean meal by 2.2 lbs of dry matter, a reduction in urea by 0.12 lbs of dry matter,
and a reduction in Soy Plus® by 0.18 lbs of dry matter. This created 2.0 lbs of dry matter space in the diet. These dietary changes resulted in greater milk yield and milk protein yield, but were accompanied by a lower milk fat percentage.

Both diets were similar in NDF, NSC and starch content. Using Optigen® 1200 as a nitrogen source allowed the successful manipulation of rate of carbohydrate digestion. Fiber from wheat midds is digested more rapidly in the rumen compared to fiber from corn (Hoover and Miller, 1991). Wheat midds have a lower 'rumen fill' value than corn, barley and distiller’s grains (Varga et al., 1984). Replacing soybean meal and Soy Plus® (high in RUP) with wheat midds and ground corn changes the rumen fill values of these diets and the rate of starch and fiber digestion. Thus, the Optigen® 1200 diet would have a lower rumen fill value and faster rate of digestion compared to the control diet. When diets with low rumen fill were compared to diets with high rumen fill, the diets with low rumen fill resulted in greater NDF and total carbohydrate digestion, and also higher milk production (Hoover and Miller, 1991).

LACTATING DAIRY COWS: EXPERIMENT 2

In a second trial, 240 lactating dairy cows on a commercial dairy in central New York were used to evaluate performance response to Optigen® 1200. Groups were balanced for days in milk, parity and milk yield at the start of the experiment. Both groups were fed the control diet for a 15-day baseline period, followed by a treatment period lasting one month. Individual daily milk weights were recorded and DHIA Dairy One (Ithaca, NY) took milk samples every 14 days. Individual cow milk samples were analyzed for CP, fat and milk urea nitrogen by the New York DHIA Milk-Testing Laboratory (Ithaca, NY). Daily feed intake by group was measured. Orts were taken daily from the feed bunk and moisture determined using a Koster moisture tester.

The ingredient composition of the diets is shown in Table 4. Forage supplied 24.86 and 25.24 lbs of DM on control and Optigen® 1200 diets, respectively. These diets contained 47.5% and 48.26% forage on a DM basis, respectively. When Optigen® 1200 was added to the diet, the amounts of urea and soybean meal were reduced. Carbohydrates (ground corn and corn silage) replaced these protein supplements. Both diets contained 0.70 lbs of buffer per cow per day as part of the mineral supplement.

Control and Optigen® 1200 diets contained similar amounts of crude protein and net energy (Table 5). The diets differed in the amount of RUP and starch. The addition of Optigen® 1200 to the diet resulted in a 4.29% decrease in RUP content and an 2.56% increase in starch content. The RUP was reduced in the Optigen® 1200 diet. One hypothesis for this is that a controlled release nitrogen supplement like Optigen® 1200 would enhance ruminal bacterial growth and bacterial protein production.

### Table 4. Composition of experimental diets fed during Experiment 2 (DM basis).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control (lb DM/day)</th>
<th>Optigen® 1200 (lb DM/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage, 1999</td>
<td>14.35</td>
<td>14.64</td>
</tr>
<tr>
<td>Haylage, 1st cutting</td>
<td>7.81</td>
<td>7.90</td>
</tr>
<tr>
<td>Idaho hay, 1999</td>
<td>2.70</td>
<td>2.70</td>
</tr>
<tr>
<td>Whole cottonseed</td>
<td>4.14</td>
<td>3.72</td>
</tr>
<tr>
<td>Ground corn</td>
<td>13.09</td>
<td>14.02</td>
</tr>
<tr>
<td>Soybean meal, 48% CP</td>
<td>3.34</td>
<td>1.98</td>
</tr>
<tr>
<td>Feed grade urea</td>
<td>0.06</td>
<td>0</td>
</tr>
<tr>
<td>Optigen® 1200</td>
<td>0</td>
<td>0.35</td>
</tr>
<tr>
<td>Corn gluten meal, 60% CP</td>
<td>0.74</td>
<td>0.56</td>
</tr>
<tr>
<td>Blood meal</td>
<td>0.26</td>
<td>0.39</td>
</tr>
<tr>
<td>Agway Amino Plus</td>
<td>2.06</td>
<td>2.00</td>
</tr>
<tr>
<td>MolMix Prime 28 liquid supplement</td>
<td>0.96</td>
<td>0.96</td>
</tr>
<tr>
<td>Tallow</td>
<td>0.20</td>
<td>0.30</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.41</td>
<td>0.38</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.28</td>
<td>0.33</td>
</tr>
<tr>
<td>Megalac</td>
<td>0.49</td>
<td>0.57</td>
</tr>
<tr>
<td>Custom min/vit plus buffer</td>
<td>1.41</td>
<td>1.48</td>
</tr>
<tr>
<td>Total dry matter</td>
<td>52.30</td>
<td>52.28</td>
</tr>
</tbody>
</table>

### Table 5. Nutrient composition of diets fed during Experiment 2 (DM basis).

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Control</th>
<th>Optigen® 1200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage, 1999</td>
<td>14.35</td>
<td>14.64</td>
</tr>
<tr>
<td>Crude protein, %</td>
<td>18.53</td>
<td>18.56</td>
</tr>
<tr>
<td>Degradeable protein, % DM</td>
<td>11.98</td>
<td>12.13</td>
</tr>
<tr>
<td>Soluble protein, % of CP</td>
<td>33.26</td>
<td>33.19</td>
</tr>
<tr>
<td>Undegradable protein, % of CP</td>
<td>34.98</td>
<td>33.48</td>
</tr>
<tr>
<td>NFC:DIP ratio¹</td>
<td>3.24</td>
<td>3.26</td>
</tr>
<tr>
<td>Net energy, mcal/lb</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td>ADF, %</td>
<td>19.41</td>
<td>19.24</td>
</tr>
<tr>
<td>NDF, %</td>
<td>28.50</td>
<td>28.28</td>
</tr>
<tr>
<td>Forage, % of DM</td>
<td>47.50</td>
<td>48.26</td>
</tr>
<tr>
<td>NFC, %</td>
<td>38.80</td>
<td>39.51</td>
</tr>
<tr>
<td>Starch, %</td>
<td>30.79</td>
<td>31.60</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.95</td>
<td>0.95</td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>0.49</td>
<td>0.49</td>
</tr>
<tr>
<td>Potassium, %</td>
<td>1.45</td>
<td>1.45</td>
</tr>
<tr>
<td>Magnesium, %</td>
<td>0.33</td>
<td>0.33</td>
</tr>
<tr>
<td>Sodium, %</td>
<td>0.61</td>
<td>0.61</td>
</tr>
<tr>
<td>Sulfur, %</td>
<td>0.28</td>
<td>0.30</td>
</tr>
</tbody>
</table>

1Estimated values based on forage analysis and diet composition.
2Non-fibrous carbohydrate:degradable intake protein.
3Acid detergent fiber.
4Neutral detergent fiber.
Daily milk yield data were analyzed using Proc Mixed Model procedure of SAS (1999). Daily observations were treated as repeated measures. Fixed effects in the SAS model included parity, treatment, stage of lactation, test day, and the interaction of treatment × stage of lactation, treatment × test day, and treatment × parity. Cow was considered as a random effect in this model. Daily DM intake by group was analyzed using the Proc Mixed Model procedure of SAS (1999). Fixed effects in this model were treatment and date. Daily observations by group were treated as repeated measures.

Delivering a part of the total dietary nitrogen in the form of Optigen® 1200 had a significant effect on DM intake but not on milk yield (Table 6). Dry matter intake was 1.97 lbs lower in the Optigen® 1200 group compared to controls. Milk fat percentage was greater on the Optigen® 1200 treatment, but fat yield was unaffected. Milk protein percentage was greater in the control group while protein yield was unaffected.

Using Optigen® 1200 as a rumen nitrogen source increased rumen efficiency. Efficiency of milk production (lb milk/lb DMI) was greater for cows given Optigen® 1200 (1.75 vs. 1.68). This represents an improvement in efficiency of 4.2%.

Indirect evidence for an improvement in ruminal efficiency can be seen when actual intake of nutrients is calculated based on actual DM intake and diet composition (Table 7). The cows receiving Optigen® 1200 consumed 3.58% less protein per day and 7.89% less RUP per day compared to controls. Since both milk yield and milk protein yield were similar, the use of Optigen® 1200 likely increased the supply of bacterial protein passing into the small intestine.

Optigen® 1200 was substituted in the diet for soybean meal, urea and whole cottonseed. Feeding 0.35 lbs of Optigen® 1200 allowed a reduction in soybean meal by 1.36 lbs of dry matter, a reduction in urea by 0.06 lbs of dry matter, and a reduction in whole cottonseed by 0.42 lbs of dry matter. This created 1.84 lbs of dry matter space in the diet. The addition of forage (0.38 lbs of DM) and ground corn (0.93 lbs of DM) filled this additional space. These dietary changes did not result in greater milk yield, but did increase milk fat percentage. The lack of milk yield response can be explained by the fact that non-fibrous carbohydrate and starch intake were lower in the Optigen® 1200 diet compared to the control diet (Table 7). The increase in milk fat percentage, however, cannot be explained by greater forage intake. Forage intake was actually lower for the Optigen® 1200 diet due to the lower dry matter intake (Table 7). The increase in fat percentage may be explained by greater forage digestion, which could have occurred due to greater ruminal bacterial activity in the Optigen® 1200 diet. Since the intake of RUP was 7.89% less in the group given Optigen® 1200 while milk yield was similar, bacterial protein production in the rumen must have increased by 7.89%. Enhanced bacterial growth and microbial protein production in response to Optigen® 1200 is a likely explanation for the improvement in efficiency of milk production (4.2%).

Table 6. Least square means of milk yield and percentage of milk fat and protein in Experiment 2.

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>SE</th>
<th>Optigen® 1200</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM intake, lb/d</td>
<td>52.74</td>
<td>0.20</td>
<td>50.77</td>
<td>0.20</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Milk yield, lb/d</td>
<td>88.51</td>
<td>0.84</td>
<td>89.00</td>
<td>0.85</td>
<td>&gt; 0.50</td>
</tr>
<tr>
<td>Milk fat, %</td>
<td>3.76</td>
<td>0.06</td>
<td>3.93</td>
<td>0.07</td>
<td>0.065</td>
</tr>
<tr>
<td>Fat yield, lb/d</td>
<td>3.41</td>
<td>0.04</td>
<td>3.55</td>
<td>0.04</td>
<td>0.268</td>
</tr>
<tr>
<td>Milk protein, %</td>
<td>3.02</td>
<td>0.02</td>
<td>2.96</td>
<td>0.03</td>
<td>0.087</td>
</tr>
<tr>
<td>Protein yield, lb/d</td>
<td>2.67</td>
<td>0.04</td>
<td>2.63</td>
<td>0.04</td>
<td>&gt; 0.50</td>
</tr>
</tbody>
</table>

Conclusion

Optigen® 1200 has an in situ nitrogen disappearance rate very similar to soybean meal. Bacterial protein synthesis and disappearance of nutrients in continuous culture fermentors were greater when Optigen® 1200 was present in the diet. Optigen® 1200 increases the crude protein density of the protein fraction of the diet because it is a highly concentrated source of nitrogen. By increasing diet protein density, space is created in the formulation, which allows nutritionists to manipulate the carbohydrate fraction of the diet. These manipulations resulted in greater milk yield and milk component yield including greater milk fat percentage. In both in vivo experiments, the amount of RUP was reduced in the Optigen® 1200 diets compared to the control diets without a loss in milk yield. These results indicate that Optigen® 1200 can be used successfully in dairy cattle diets to create
space for more rumen degradable carbohydrate. A combination of improved bacterial protein synthesis and availability of greater concentrations of degradable carbohydrate makes Optigen® 1200 a useful tool for enhancing milk yield.

References


Dairy nutrition models: their forms and applications

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Introduction

For some years it has been evident that dairy cow nutrition models are vital to the continued success of the dairy industry. This is especially true as we recognize the importance, for example, of ruminal microbes and metabolism in body tissues to nutrient requirements. In addition, our production emphasis has shifted from only milk volume and fat to include milk protein percentage and yield. Mathematical models of nutrition have been in use for over three decades and have stimulated improvement in feeding cattle. However, more complete data sets available in recent years combined with more precise mathematical approaches have now allowed us to improve models of nutrient use tremendously. Such models will be used more frequently in the future for support of decisions not only on the nutrition of cattle, but for other aspects including farm economics and environmental impact.

Dairy nutrition models: forms and roles

Nutritional models vary in complexity according to objectives. A typical scheme of model levels needed to represent a system is found in Table 1. Information about a system must be at least one level below the system explored with the model. Thus, models describing herds operate at the animal level or below, those describing animals require details at the organ level and lower and so on.

In practice, models only need details that have significant bearing on consequences of changes arising from inputs to the system (Production Model) or as much detail as is necessary to explore the system in new and different ways (Scientific Model). Salient properties of production and scientific models are presented in Table 2.

Table 1. Model levels.

<table>
<thead>
<tr>
<th>Level</th>
<th>Description of level</th>
</tr>
</thead>
<tbody>
<tr>
<td>i+1</td>
<td>Collection of organisms (herd, flock, crop)</td>
</tr>
<tr>
<td>i</td>
<td>Organism (animal, plant)</td>
</tr>
<tr>
<td>i-1</td>
<td>Organs</td>
</tr>
<tr>
<td>i-2</td>
<td>Tissues</td>
</tr>
<tr>
<td></td>
<td>Cells</td>
</tr>
<tr>
<td></td>
<td>Organelles</td>
</tr>
</tbody>
</table>

1Adapted From France and Thornley (1984).

Scientific models are usually developed upward from basic experimental data pertaining to metabolic processes. Scientific models assume that a living system can be described in terms of a set of ‘critical’ metabolic transactions encapsulated in organs. The goal is to translate in vitro experimental data into chemical reactions representing the essential metabolic processes. Differential equations of the mass balance and Michaelis Menten forms are used to describe substrate level changes as the system equilibrates to a (new) steady state because of nutritional and digestive inputs. Implicit to these models are two basic assumptions: firstly, that in vivo metabolic pathways can be represented using the critical transactions modeled from in vitro experimental data, and secondly, that cellular level metabolic processes can be aggregated to the organ level to effectively model whole animal function. Baldwin, at the University of California, and his colleagues (Baldwin...
et al., 1987a,b,c) have produced a comprehensive integrated model that describes digestion and metabolism of the dairy cow with dynamic, mechanistic equations of physiological processes.

**PRODUCTION MODELS**

Production models are primarily used to portray animal responses to different inputs. They are usually created from collections of response surface models that are developed from animal or herd level experiments. Thus, these models are developed downward. They are valid within the domain of data underpinning the individual response surfaces and are as accurate as the response models themselves.

A theme for the development, refinement and deployment of empirical production models is seen in the development and implementation of the National Research Council dairy cow models (NRC, 1978; 1989; 2001). In 1978, response equations were used to predict crude protein and energy needs of the dairy cow. The 1989 model used a system of protein utilization that partitioned dietary protein into rumen degradable (DIP) and rumen undegradable (UIP) fractions (NRC, 1985). Growth of microorganisms in the rumen was driven by energy intake (TDN, NE$_{l}$). In 2001, the National Research Council released a new dairy cow model that contains some of the mechanistic approaches in the CNCPS/CPM-Dairy that are described below.

Other empirical production models include the VEM-DVE/OEB (Dutch; Tamminga et al., 1994), AFRC (British; AFRC, 1990; 1992), CSIRO (Australian; CSIRO, 1990) and INRA (French; INRA, 1989) systems. These early production models stimulated more precise thinking and experimentation. Better data were incorporated into newer versions of models. Largely because of concepts in these increasingly precise models, rations for dairy cows usually now contain feed ingredients that are resistant to ruminal degradation. This increases overall efficiency of dairy cow feeding.

The need for more accurate models to define rumen bacterial and whole animal requirements, to assess feed utilization and to predict production responses led to the development of the Cornell Net Carbohydrate and Protein System (Fox et al., 1992; O’Connor et al., 1993; Russell et al., 1992; Sniffen et al., 1992). The CNCPS is a mix of empirical and mechanistic approaches that describe feed intake, ruminal fermentation of protein and carbohydrate, intestinal digestion and absorption, excretion, heat production, and utilization of nutrients for maintenance, growth, lactation and pregnancy. When the CNCPS was evaluated with data from individual dairy cows where the appropriate inputs were measured and changes in energy reserves were accounted for, 90% of the variation in actual milk production of individual cows was explained with a 1.3% bias. The model accounted for 76% of the variation in individual cow milk production with an 8% underprediction bias when energy was first limiting and 84% of the variation with a 1.1% overprediction bias when protein was first limiting (Fox et al., 2004).

### Dairy nutrition software

Dairy nutrition models often do not contain tools for computer assisted ration formulation. Software
included with the 1989 and 2001 NRC dairy nutrition models allowed calculation of nutrient requirements and evaluation of rations but did not provide for formulation of rations.

AUTO-BALANCING

The usual objective of auto-balancing is to produce an ‘optimal ration’ at the lowest cost. Constraints (minimum and maximum amounts) are set for both nutrients and feed ingredients. Nutritional constraints describe the requirements of cows to perform specific or multiple functions (maintenance, growth, lactation, pregnancy). Nutritional constraints include dry matter intake, energy (metabolizable and net), protein (crude, soluble, bypass and metabolizable), carbohydrates (fiber and non-fiber), fat, minerals; and in the case of newer models like the CNCPS/CPM-DAIRY, amino acids and rumen available nitrogen (peptides and ammonia) are included. Feed ingredients are selected on the basis of the major nutrients that they provide (i.e. fiber from forages, non-fiber carbohydrates from grains, protein from oilseed meals). Feed constraints are set based on the availability of purchased ingredients and inventory of ingredients on the farm or contracted for purchase. The amount of an ingredient specified is often adjusted by the formulator to take into account a minimum amount that the formulator feels rations should contain or the maximum amount that the formulator feels can be tolerated by the animal. The amount of a feed ingredient should not be limited by high cost because optimization programs will control the inclusion of expensive feeds. Thus, the auto-balancing (optimization) task is to find the least cost combination of feed ingredients within their minimum and maximum constraints that provide nutrients that are within the specified minimum and maximum ranges. When the foregoing is achieved, the auto-balancing process has provided a solution to the specifications defined by the formulator.

Ration formulators often are discouraged when the optimization process does not give a solution as defined above. This provided no direction to obtaining a solution. Newer optimization methods provide direction by listing nutrient constraints that are not met.

In both empirical models and in the CNCPS/CPM-DAIRY, nutrients like crude protein, fat, carbohydrates (fiber and non-fiber) and minerals are constant proportions of the ingredient regardless of the amount of feed consumed. Thus, supply of these nutrients is a linear function of intake. In empirical dairy cow models, metabolizable protein and energy (metabolizable and net) values also are not affected by intake and thus are constant. Thus linear programming can be used for auto-balancing. The CNCPS/CPM-DAIRY has a dynamic rumen sub-model wherein the passage rate of feeds (determined mainly by feed intake but also adjusted by ration forage content and particle size) determines the outflow of nutrients from the rumen system. Thus, nutrients like metabolizable protein, metabolizable energy, amino acid content of metabolizable protein, and rumen available protein (peptides and ammonia) are not constant but vary according to feed consumption and ration ingredients. These features of the dynamic digestion models mean that the problem of dairy cow ration optimization is no longer the province of linear programming and nonlinear programming packages are required. Implementing constrained, nonlinear optimization is not without problems. If the nutrition model contains discontinuous (break-point) functions, continuous mathematical models must be developed to describe the discontinuous functions (Boston et al., 2000). Whereas a linear programming problem can be solved from any starting point, a nonlinear programming problem requires a ‘good’ feasible starting point to ‘effectively’ start the solution process. Finally, a linear programming optimization problem has just one solution. This is not so for nonlinear optimization.

Ration formulation was one of the first applications of linear programming. Not only could solutions be found in seconds, but building on contributions of Dantzig (1995) to operational research, we also were able to derive an array of other very helpful economic properties (shadow prices) relating to our optimal solution. For example, we could discover the cost ranges over which feeds within the optimal ration remained there, as well as which amongst the feeds not selected in the optimal ration were candidates for inclusion if cost decreased.

Bath and Bennett (1980) at The University of California were amongst the earliest to employ linear
programming to formulate rations for maximum income over feed costs. Galligan and coworkers (1986) at the University of Pennsylvania programmed the 1978 NRC dairy nutrition model into Lotus 1-2-3 with auto-balancing of rations provided by Einfin. Spartan represented an excellent effort in software development by the group at Michigan State University that was based on NRC models and included auto-balancing (Vandehar et al., 1992).

CPM-Dairy is a combined effort by researchers at Cornell University, the University of Pennsylvania and the W.H. Miner Agricultural Research Institute (Boston et al., 2000). CPM-Dairy contains the CNCPS and is a field efficient program for use with growing, lactating and dry cows. The optimization scheme was developed by the Systems Programming Group at the University of Maryland (Zhou and Tits, 1997) and employs a forward sequential quadratic programming approach. Version 1, released in 1998, also contained a modification of NRC (1989). Versions 2 and 3 only contain the CNCPS and are in beta testing with release anticipated in 2004. Version 3 has expanded carbohydrate fractions, a lipid submodel (Moate et al., 2004) and incorporates NRC (2001) mineral requirements.

**COMMERCIALY AVAILABLE SOFTWARE**

Table 3 contains a list of commercially available software for formulation of dairy cattle rations. NRC,

<table>
<thead>
<tr>
<th>Software</th>
<th>Developers</th>
<th>Dairy Nutrition Model</th>
<th>Website</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed Ration Balancer</td>
<td>Feed Management Systems</td>
<td>NRC, User defined</td>
<td><a href="http://www.feedsys.com/">http://www.feedsys.com/</a></td>
</tr>
<tr>
<td>CamDairy</td>
<td>Cam Software</td>
<td>Proprietary model</td>
<td><a href="http://epicentre.massey.ac.nz/">http://epicentre.massey.ac.nz/</a></td>
</tr>
<tr>
<td>CPM-Dairy</td>
<td>Cornell U., U.Pennsylvania, Miner Institute</td>
<td>CNCPS</td>
<td><a href="http://mail.vet.upenn.edu/~ejjancze/">http://mail.vet.upenn.edu/~ejjancze/</a></td>
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<tr>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>cpmbeta3.html</td>
</tr>
<tr>
<td>CNCPS</td>
<td>Cornell University</td>
<td>CNCPS</td>
<td><a href="http://www.cncps.cornell.edu/cncps/main.htm">http://www.cncps.cornell.edu/cncps/main.htm</a></td>
</tr>
<tr>
<td>Dairy Ration System</td>
<td>ACS Computer Services</td>
<td>NRC</td>
<td><a href="http://www.acsdrs.com">http://www.acsdrs.com</a></td>
</tr>
<tr>
<td>Formulate2</td>
<td>Central Valley Nutritional Associates</td>
<td>NRC</td>
<td><a href="http://www.formulate2.com/">http://www.formulate2.com/</a></td>
</tr>
<tr>
<td>INRAtion - PrevAlim</td>
<td>INRA</td>
<td>INRA</td>
<td><a href="http://www.cnerta.educagri.fr/unites/lpa/lpa.htm#inration">http://www.cnerta.educagri.fr/unites/lpa/lpa.htm#inration</a></td>
</tr>
<tr>
<td>Mixit-Win</td>
<td>Agricultural Software Consultants, Inc.</td>
<td>User defined minimum and maximum nutrient amounts</td>
<td><a href="http://www.asc-mixit.com/">http://www.asc-mixit.com/</a></td>
</tr>
<tr>
<td>Molly</td>
<td>U. California, Davis</td>
<td>Molly</td>
<td><a href="http://animalscience.ucdavis.edu/research/molly/default.htm">http://animalscience.ucdavis.edu/research/molly/default.htm</a></td>
</tr>
<tr>
<td>PCDairy-2</td>
<td>U. California, Davis</td>
<td>NRC</td>
<td><a href="http://animalscience.ucdavis.edu/extension/pcdairy.htm">http://animalscience.ucdavis.edu/extension/pcdairy.htm</a></td>
</tr>
<tr>
<td>RationPro</td>
<td>ProfitSource</td>
<td>NRC, User-defined</td>
<td><a href="http://www.rationpro.com/">http://www.rationpro.com/</a></td>
</tr>
<tr>
<td>Shield</td>
<td>U. California, Davis</td>
<td>Proprietary model</td>
<td><a href="http://animalscience.ucdavis.edu/extension/shield.htm">http://animalscience.ucdavis.edu/extension/shield.htm</a></td>
</tr>
<tr>
<td>SigaDairy</td>
<td>Siga Farm Software</td>
<td>NRC, User-defined</td>
<td><a href="http://www.siga.net">http://www.siga.net</a></td>
</tr>
<tr>
<td>Spartan</td>
<td>Michigan State U.</td>
<td>NRC with modifications</td>
<td><a href="http://www.msu.edu/user/ssl/index.htm">http://www.msu.edu/user/ssl/index.htm</a></td>
</tr>
<tr>
<td>Trilogic</td>
<td>Trilogic Systems</td>
<td>NRC, User-defined</td>
<td><a href="http://trilogic-systems.com/">http://trilogic-systems.com/</a></td>
</tr>
</tbody>
</table>

N.RC, National Research Council; CNCPS, Cornell Net Carbohydrate and Protein System; INRA, Institut National de la Recherche Agronomique, MOLLY, a dynamic, mechanistic computer model of a dairy cow; AFRC, Agricultural and Food Research Council; PDI (INRA), Protéines variés reellement digestibles dans l’Intestin grele.
INRA and CNCPS dairy nutrition models are used in some of the software packages whereas proprietary or user-defined models are used in others. Linear programming is used for auto-balancing in empirical models. In CNCPS, biological values for metabolizable energy, metabolizable protein, passage rate, bacterial yield efficiencies and degradation rate of available fiber, which depend upon feed intake and the ingredients selected, are first estimated and then rations are balanced using linear programming. In CPM-Dairy, a nonlinear optimizer is used to auto-balance rations according to the CNCPS (Zhou and Titts, 1997).

Application of dairy nutrition models

CPM-Dairy is used by veterinarians, nutrition consultants and the feed industry to evaluate and formulate rations for dairy cattle.

At New Bolton Center, our Field Investigation Unit was presented with a case where milk production in a 200-cow dairy was only 30 kg/d although cows were milked three times daily and were treated with bST. Early lactation cows frequently went off feed and had diarrhea. Feces contained undigested corn, believed to be high moisture corn.

There were two high production groups (heifers and high cows) and a low production group. The heifers and high production cows were housed in a new free-stall barn with excellent ventilation and cow comfort. Cows were fed three times daily with frequent ‘push-up’ of feed. Feed bunk space was 1.7 feet per cow with no headlocks, and cows had good water access. Remaining milking cows were housed in a renovated free-stall barn. They were fed twice daily with frequent ‘push-up’ of feed. Feed bunk space and ventilation were good. Non-lactating cows were housed on a bedded pack.

CPM-Dairy was used to evaluate the existing rations and to formulate new rations. In Table 4 are details of pre-CPM-Dairy and CPM-Dairy rations for the high producing animals (heifers and high cows).

The pre-CPM-Dairy ration was formulated for a target of 39 kg/d milk with 3.6% fat and 3.1% crude protein. According to CPM-Dairy, this ration was low in metabolizable protein and would only support 34.4 kg/d milk. Poor amino acid ratios (Met/MP=1.89; Lys/MP=6.24) could reduce milk production by 1.6 kg/d so that expected milk on the basis of metabolizable protein and balance of amino acids was only 32.8 kg/d. The ration was marginal in eNDF (21% vs the guideline of 23% of ration DM) and contained 41.5% non-fiber carbohydrate (NFC). High moisture corn, which has an initial high rate of fermentation in the rumen, contributed a substantial amount of NFC.

Table 4. Field application of CPM-Dairy.

<table>
<thead>
<tr>
<th>Ingredients (%DM)</th>
<th>Pre-CPM-Dairy</th>
<th>CPM-Dairy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa silage</td>
<td>27.14</td>
<td>15.21</td>
</tr>
<tr>
<td>Corn silage</td>
<td>22.79</td>
<td>37.16</td>
</tr>
<tr>
<td>High moisture corn</td>
<td>24.23</td>
<td>18.95</td>
</tr>
<tr>
<td>Soybean hulls</td>
<td>6.92</td>
<td>5.63</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>3.91</td>
<td>5.51</td>
</tr>
<tr>
<td>Corn distiller's grains</td>
<td>3.34</td>
<td>5.51</td>
</tr>
<tr>
<td>Dry brewer's grains</td>
<td>9.29</td>
<td>10.00</td>
</tr>
<tr>
<td>Whole cotton seed</td>
<td>1.38</td>
<td>0.55</td>
</tr>
<tr>
<td>Fish meal</td>
<td>3.82</td>
<td>3.38</td>
</tr>
<tr>
<td>Blood meal</td>
<td>0.40</td>
<td>0.49</td>
</tr>
<tr>
<td>Animal-marine protein blend</td>
<td>0.59</td>
<td>0.49</td>
</tr>
<tr>
<td>Megalac</td>
<td>0.59</td>
<td>0.40</td>
</tr>
<tr>
<td>Megalac Plus</td>
<td>0.40</td>
<td>0.59</td>
</tr>
<tr>
<td>Salt</td>
<td>0.50</td>
<td>0.80</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0.50</td>
<td>0.58</td>
</tr>
<tr>
<td>Mineral and vitamin mix</td>
<td>1.03</td>
<td>1.03</td>
</tr>
<tr>
<td>Total dry matter, kg/d</td>
<td>22.46</td>
<td>22.46</td>
</tr>
<tr>
<td>Cost, $/day</td>
<td>3.65</td>
<td>3.82</td>
</tr>
</tbody>
</table>

Carbohydrates
Non fiber1, %DM 41.5 39.0
Neutral detergent fiber, %DM 30.4 33.3
Effective neutral detergent fiber, %DM 21.0 22.9

Protein
Crude, %DM 18.6 17.8
Undegraded, %CP 33.7 37.2
Metabolizable, kg/d 2.28 [100%] 2.49 [100%]
Bacterial 1.25 [55%] 1.34 [54%]
Undegraded 1.03 [45%] 1.15 [46%]

Metabolizable amino acids
Methionine, g/d 43 55
Met/Mp 1.89 2.19
Lysine, g/d 142 173
Lys/Mp 6.24 6.94

Nutrient limited milk, kg/d
Metabolizable energy 44.0 43.8
Metabolizable protein 34.4 39.1
Rulquin Ratio1 32.8 39.0

1. Includes sugars, starch, pectin, B-glucans and acids produced during silage fermentation. Silage acids are not fermented further in the rumen and do not provide energy for bacterial growth.
2. Values in brackets are percentages of metabolizable protein
3. Responses to amino acid ratios calculated according to equations in Rulquin and Verite (1993) and added to metabolizable protein-limited milk.

Objectives in formulating the new ration were to 1) provide less NFC without compromising total carbohydrate fermentability, 2) increase eNDF, 3) correct the deficiency of metabolizable protein, and 4) improve amino acid balance.
Reducing amounts of high moisture corn (19 vs 24% DM) and alfalfa silage (15 vs 27% DM), increasing the amount of corn silage (37 vs 23% DM) and including soybean hulls (7 vs 0% DM) reduced NFC (39.0 vs 41.5%). Although NFC was lower, total fermentable carbohydrates were higher (45 vs 43% ration DM) because of the high ruminal fermentation of NDF in soybean hulls and corn silage. Increasing ration forage (52 vs 50% DM) and including soybean hulls increased NDF (33.3 vs 30.4%) and eNDF (22.9 vs 21.0%).

Replacing a portion of the soybean meal and all of the corn distiller’s grains and dried brewer’s grains with animal and marine proteins increased the supply of metabolizable protein from rumen undegraded protein by 0.12 kg (1.15 vs 1.03 kg/d). Metabolizable protein from ruminal bacteria was increased (1.34 vs 1.25 kg/d) for two reasons. First, bacterial growth is driven mainly by energy derived from fermentation of carbohydrates. As noted above, the CPM-Dairy ration contained more rumen fermentable carbohydrates (45 vs 43 % DM, 10.05 vs 9.76 kg/d) than the pre-CPM-Dairy ration. Second, bacterial growth is decreased when rumen pH decreases. The CPM-Dairy ration contained more eNDF than the pre-CPM-Dairy ration. This stimulates cud chewing, increases salivary flow and improves ruminal buffering. More metabolizable protein from rumen escape protein and from bacterial protein alleviated the deficiency of metabolizable protein so that milk yield based on metabolizable protein provided by the CPM-dairy ration was 39.1 kg/d compared to 34.4 kg/d for the pre CPM-dairy ration.

Animal and marine proteins and ruminal bacteria are excellent sources of lysine. Thus, the combination of ruminal escape and bacterial protein improved Lys/MP (6.94 vs 6.24). Fish meal, but not blood meal nor rumen bacteria are good sources of methionine. Met/MP (2.19 vs 1.89) was improved by using Megalac Plus (Alimet in calcium salts of LCFA) to supplement methionine in rumen escape and bacterial protein. Amino acid balance of the ration would only reduce milk 0.1 kg/d compared to 1.6 kg/d with the pre CPM-Dairy rations. Thus, on the basis of metabolizable protein supply and amino acid balance, expected milk was 39 kg/d or 6.2 kg/d more than on the pre CPM-dairy ration.

After one week on the new high production rations and a similarly formulated low production ration, total herd milk production was 4 kg/d higher. Cud chewing increased and manure consistency improved. Off-feed problems were dramatically reduced.

Two weeks after the ration change, manure still contained excessive undigested high moisture corn. Grindling the high moisture corn through a 0.635 cm screen reduced corn in feces and milk production increased another 2 kg/d. Herd milk production now averaged 36 kg/d. For the next 12 months, average milk production for the total herd was 36 to 38 kg/d.

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Gastrointestinal development in dairy calves

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Gastric enzymes in the dairy calf

The role of gastric enzymes is important for the digestion of the diet of the young ruminant animal due to its undeveloped rumen and reliance on the abomasum and small intestine for nutrient digestion and assimilation. The ability of neonates to digest and utilize high concentrations of milk fat, especially with low concentrations of intestinal lipases, is due to a combination of enzymes called pregastric esterase (Huber et al., 1961). This complex of lipolytic enzymes provides the majority of lipid breakdown within the abomasum, similar to salivary α−amylase in monogastrics. Pregastric esterase is composed of at least six different enzymes secreted from four areas of the glosso-epiglottic area of the mouth, including the vallate papillae region of the tongue, the glosso-epiglottic area, the pharyngeal end of the esophagus and the submaxillary salivary gland (Moreau et al., 1988; Ramsey et al., 1956). It is of interest to note that adult ruminants in general are not capable of digesting diets high in fat, yet it is one of the main components in the diet of a neonatal ruminant.

Although the term pregastric esterase is commonly used to describe esterases (defined as enzymes capable of hydrolyzing esters in solution) it also contains lipases, which have a more specific role in digestion (defined as specialized enzymes that hydrolyze fatty acids from water insoluble glycerol esters). Pregastric esterase has become the common term for enzymes of lipolytic or esterolytic nature secreted by mammalian oral tissues (Nelson et al., 1960).

Pregastric esterase is stimulated by nursing in the young calf (Huber et al., 1961; Moreau et al., 1988; Ramsey and Young, 1961a). During clotting of milk that occurs after ingested milk reaches the abomasum in young ruminants, pregastric esterase begins the breakdown of lipids within the casein clot (Bondi, 1987; Hill et al., 1970). Pregastric esterase hydrolyzes about 20% of all milk fat glyceride linkages, mainly short chain fatty acids, indicating its role in lipolysis (Bondi, 1987; Hill et al., 1970; Pitas and Jensen, 1970).

The preference of pregastric esterase for glyceride linkages of butyric acid has been reported to result in 59% of butyrate linkages hydrolyzed in vitro as compared with 7% of higher fatty acids (Ramsey and Young, 1961b). Butyric acid is highly soluble in water and is hydrolyzed faster than longer chained fatty acids, passing quickly from the abomasum to the small intestine. Breakdown of butyric acid and other fatty acids is higher in milk fed orally than milk that is infused into the abomasum, indicating pregastric esterase is required (Otterby et al., 1964a; Otterby et al., 1964b; Russell et al., 1980). Other less soluble fatty acids are trapped by curd particles and slowly migrate to the small intestine (Otterby et al., 1964a; Ramsey and Young, 1961a). Although pregastric esterase plays a primary role in lipolysis in the abomasum, calves fed only through infusion of milk into the abomasum, which decreases stimulation of pregastric esterase secretion, are still able to digest milk fat most likely by pancreatic lipase action (Russell et al., 1980).

Pregastric esterase may also be found in the digesta of the small intestine, which was thought to indicate a role in intestinal digestion of lipids (Otterby et al., 1964b). Later work reported that pregastric esterase has a diminished effect once it enters the duodenum and no effect at all if pancreatic enzymes are not present (Gooden, 1973).

A small part of lipolysis in the abomasum was reported to be caused by a gastric lipase secreted directly from abomasal tissues. Toothill et al. (1976)
used abomasal pouch secretions to prevent oral or pancreatic contamination and found no lipolytic digestion, concluding that lipolysis in the abomasum is solely due to pregastric esterase. Other researchers also concluded that gastric lipase is similar to pregastric esterase and must have been mistakenly identified as a separate enzyme (Nelson et al., 1960; Otterby et al., 1964b).

Although most of the enzymes in the alimentary tract of the young calf seem underdeveloped, enzymes that primarily coagulate milk protein are produced in high concentrations. Chymosin, pepsin and hydrochloric acid coagulate milk, retaining casein and fat and allowing nutrients to slowly pass into the small intestine (Cruywagen et al., 1990; Guilloteau et al., 1983; Guilloteau et al., 1984). Chymosin, formerly called rennin, is found in high concentrations in the newborn calf and lamb and decreases with age and at weaning (Cybulski and Andren, 1990; Guilloteau et al., 1983; Guilloteau et al., 1984; Guilloteau et al., 1985). However, calves kept on milk for an extended period of time retain higher chymosin concentrations than weaned calves indicating abomasal enzymes are regulated by development of the rumen as well as high concentrations of lipids and casein in the diet (Cybulski and Andren, 1990; Guilloteau et al., 1985).

The bovine abomasum secretes at least three proteases, including pepsin A, pepsin B (also known as gastricsin or pepsin II), and chymosin, all secreted as proenzymes from the mucous cells of the pyloric and fundic glands and requiring a pH of less than 4 to become active (Cybulski and Andren, 1990). Pepsin A is found in high concentrations at birth and remains constant with increasing age of both calves and lambs (Guilloteau et al., 1983; Guilloteau et al., 1984; Guilloteau et al., 1985), until about 44 days (Huber et al., 1961). After weaning, the ratio of pepsin to chymosin increases the need to digest protein in solid feed rather than casein (Cybulski and Andren, 1990; Guilloteau et al., 1983; Guilloteau et al., 1985). Pepsin A and chymosin adequately coagulate milk in the abomasum of the young calf, and therefore pepsin B is not found until weaning, when concentrations of chymosin decrease and the pH of the abomasum acquires a broader range due to varied feed (Cybulski and Andren, 1990). The potential for pepsin, trypsin, chymotrypsin and amylase to be secreted from the pancreas increases as the amounts of starch and protein increase in the diet (Garnot et al., 1977; Guilloteau et al., 1985). Therefore these generally increase with age and increasing dry matter intake of grains in the diet.

### Pancreatic enzymes

During the first two days of life, high concentrations of abomasal enzymes clot colostrum and allow immunoglobulins to pass into the small intestine. Pancreatic enzymes are found in low concentrations at birth until two days of age in young ruminants, allowing immunoglobulins to remain intact. It is not until after two days of age that concentrations begin to increase until around 42 days of age (Guilloteau et al., 1983; Guilloteau et al., 1984).

Pancreatic amylase is a glycosidic enzyme found in the small intestine, making up 5-6% of total protein in human pancreatic secretions (Lowe, 1994). However, in ruminants, only 2% of total protein in pancreatic secretions is α-amylase indicating a decreased intestinal ability to hydrolyze starch (Keller et al., 1958). Starch is not part of the diet of milk-fed ruminants and mature ruminants are able to hydrolyze starch in the rumen. Pancreatic amylase is found at low levels in the newborn and increases with age (Guilloteau et al., 1983; Huber et al., 1961; Le Huérou et al., 1992; Morrill et al., 1970).

Pancreatic fluid also contains two nuclease, deoxyribonuclease I (DNase) and ribonuclease (RNase). Although there is little known about either, RNase is required in weaned calves to recover phosphorus from bacterial RNA (Lowe, 1994).

Unlike other pancreatic enzymes, all peptidases are secreted aszymogens or proenzymes. The main activator of pancreatic peptidases, trypsinogen, is also secreted as a proenzyme and is cleaved by enteropeptidase to the active form of trypsin. Trypsin then cleaves other proenzymes, including chymotrypsinogen, procarboxypeptidase A, procarboxypeptidase B and procolipase, to their active forms of chymotrypsin, carboxypeptidase A and B and colipase (Lowe, 1994). Trypsin amounts secreted in pancreatic juice are low in the newborn ruminant and increase with age during the first two to four weeks in both the lamb and the calf. Chymotrypsin is higher in the young animal than trypsin but the ratio decreases with age (Guilloteau et al., 1983; Guilloteau et al., 1984; Huber et al., 1961).

Pancreatic lipases, such as colipase and phospholipase A, are low at birth and then increase and remain constant (Gooden, 1973; Guilloteau et al., 1984; Huber et al., 1961; Le Huérou et al., 1992). These lipases are highly active at a pH of 8.5 and specifically hydrolyze triglycerides and phospholipids. Guilloteau et al. (1984) reported that from birth until three weeks of age, the colipase/lipase ratio is higher than one indicating pancreatic
activity is entirely expressed in pancreatic juice in the intestinal lumen. Although calves one to two weeks of age have a diminished capacity to absorb lipids if pancreatic enzymes are removed, there still remains lipolytic activity in the intestinal lumen (Gooden, 1973; Gooden and Lascelles, 1973). This is most likely due to enzymes present in the brush border of intestinal villi.

**Brush border enzymes**

There are many peptidases found in the microvilli of enterocytes. The main hydrolases found in the brush border are aminopeptidase N, aminopeptidase A, and dipeptidyl peptidase IV (Le Huérou-Luron, 2002). All of these peptidases are designed to cleave specific terminal amino acids from proteins (Palmer, 1995). Aminopeptidase N hydrolyzes peptides in a stepwise manner up to a certain point at which dipeptidyl peptidase IV finishes the hydrolysis (Le Huérou-Luron, 2002). Aminopeptidase A, aminopeptidase N, and alkaline phosphatase are highest in the calf until two days of age and then decrease until one week of age. They remain constant until weaning, at which time they increase (Le Huérou et al., 1992).

There are four major disaccharidases found in the brush border of the small intestine including maltase-glucosidase, sucrase-isomaltase, lactase, and trehalase (Le Huërou-Luron, 2002). Sucrase-isomaltase is not present in cattle and addition of sucrose to the diet of young ruminants causes an increase in scouring (Huber et al., 1961; Le Huërou et al., 1992; Le Huërou-Luron, 2002). Maltase-glucosidase hydrolyzes α(1-4) glucosidic bonds whereas isomaltase hydrolyzes α(1-6) glucosidic bonds (Le Huërou-Luron, 2002). Maltase concentrations are low at birth and increase between 7 and 119 days after birth. Isomaltase is at low concentrations in the newborn and increases after weaning (Le Huérou et al., 1992). Trehalase, one of the four disaccharidases, is able to hydrolyze trehalose to produce free monomeric glucose for absorption (Le Huërou-Luron, 2002).

Lactase is the only enzyme capable of β(1-4) glucosidase and β(1-4) galactosidase activity. It is able to break down lactose to glucose and galactose as well as cellobiose to two monomers of glucose (Le Huërou-Luron, 2002). Lactase activity is highest at birth and decreases until about three weeks of age (Huber et al., 1961) or one week of age (Le Huérou et al., 1992) and then remains constant until levels drastically decline at weaning. However, feeding a diet high in lactose does not alter lactase concentrations (Huber et al., 1961).

Although digestive enzyme concentrations are dependent on age of the calf, early researchers reported that digestive enzyme secretions are not affected by diet of the calf (Guilloteau et al., 1983; Guilloteau et al., 1984; Huber et al., 1961; Young et al., 1960). More recent work has found alterations in secretions of digestive enzymes when different sources of protein are used in milk replacers. Unprocessed soy protein in milk replacers often alters intestinal morphology by decreasing absorptive surface area of intestinal villi as well as concentration of disaccharidases in the brush border (Pederson and Sissons, 1984). Trypsin and chymotrypsin secretions are also inhibited by allergens and anti-trypsin factors in soy protein when ingested by the young calf (Gorrill et al., 1967; Guilloteau et al., 1986; Mir et al., 1991). Soy protein may be included in milk replacer fed to calves older than 20 days of age in low concentrations but must be processed to decrease concentrations of potential allergens as well as increase protein digestibility (Mir et al., 1991).

Digestibility of milk replacer is affected by other additions, such as using high concentrations of starch as alternative energy sources. Incorporating starch into milk replacer for preruminants is not recommended due to low concentrations of pancreatic amylase present in the intestine. Starch contains 17-30% amylose and 70-83% amylopectin. Amylose consists of glucose units arranged in a linear polymer bond with α(1,4) glycosidic linkages and amylopectin is bound by α(1,6) branch points along the α(1,4) linked glucose polymer. These bonds must be broken before absorption can occur and the low concentrations of pancreatic amylase present do not hydrolyze enough bonds to provide adequate energy for the calf (Morrill et al., 1970).

However, once the calf is older and the rumen is more developed, starch becomes a primary part of the diet. Development of the rumen including formation and elongation of rumen papillae is critical in transforming the monogastric calf to the ruminant dairy heifer (Church, 1988). More economical diets and reduced labor required for feeding are the driving forces to demand this change as early as possible. The primary limiting factor for weaning dairy calves from milk or milk replacer is having adequate development of the rumen in order to maintain post-weaning growth at desired rates.
Rumen development is stimulated by the presence of microbial produced volatile fatty acids, primarily butyric acid (Beharka et al., 1998; McLeod and Baldwin, 2000). Volatile fatty acids introduced into the rumen as purified sodium salts, especially sodium butyrate, increase epithelial development (Tamate et al., 1962). Butyric acid provides energy for the rumen wall for maintenance and development. This development includes the formation of papillae and thickening of the rumen wall, including increased capillary development (Weigand et al., 1975). Any additional butyric acid that is not needed as energy for the rumen is transported into the blood stream as β-hydroxybutyrate. Around 90% of butyrate produced in the rumen is directly absorbed (Bergman, 1990; Weigand et al., 1975). Rumen wall growth and capillary development are needed to increase the ability of this organ to absorb rumen produced energy substrates. Since the microbial end products of starch are propionic and butyric acids, rumen development is most influenced by ingestion of grain and the digestion of the starch components in the grain. When starch is broken down in the rumen, volatile fatty acids (VFAs) are produced. (Greenwood et al., 1997; Krehbiel et al., 1992; Nocek et al., 1984; Weigand et al., 1975).

In the young pre-ruminant calf, the ability to begin lowering the pH helps provide an environment for the continuation of a normal rumen bacterial and protozoan population. Any way to augment the process of the digestion of starch may allow for faster breakdown of starch by bacteria into propionic acid for absorption as energy and butyric acid to stimulate development of the rumen. Addition of exogenous α-amylase may be one way to increase starch digestion in the ruminant animal. α-Amylase hydrolyzes α(1,4) glycosidic linkages but is unable to hydrolyze α(1,6) bonds in the branch points. Because of this, α-amylase only begins starch breakdown and the rest is continued through microbial digestion.

Thus, it was of interest to determine whether the addition of two levels of amylase (Amaize™, Alltech Inc.) supplied in calf starter could have an effect on rumen development by increasing the initial rate of starch digestion in the developing rumen.

A study of the effects of added amylase in Amaize™

Recently Gehman et al. (2003) utilized fifteen Holstein bull calves to study effects of calf starter supplemented with 0, 6, and 12 g/h/d of amylase as supplied by Amaize™. Calves were assigned to a treatment group on arrival with a total of five calves per treatment group. Calves were fed milk replacer (Land O’ Lakes 20% all-milk protein, 20% fat product), at a rate of 10% of arrival body weight twice per day for the duration of the trial. Calves were fed calf starter (18% CP, Purina Mills, Inc.) and water once a day on an ad libitum basis. Grain was fed in the morning after milk feeding and was done in such a way that the first 200 g/d of grain contained the treatment dosage (6 or 12 g/d) of Amaize™. After calves ate this amount each day they were offered ad libitum grain for the remainder of the day. This was done so that calves were receiving the treatment at the desired rate of daily intake at the earliest possible age. Fecal and health scores were monitored daily. Calves were euthanized at the end of 5 weeks (35 days) of age. The reticulorumen were sampled in nine areas for papillae analysis as shown in Figure 1.

Samples were taken from the rumen from the following regions: the caudal portion of the caudal ventral blind sac (A), the right and left caudal dorsal blind sac (RB and LB), the right and left cranial dorsal blind sac (RC and LC), the right and left cranial ventral blind sac (RD and LD), and the right and left caudal ventral blind sac (RE and LE). The rumen papillae were measured according to length, width, papillae per cm², and rumen wall thickness (Lesmeister et al., 2002). All 15 calves arrived healthy, there was no mortality, and no calves were treated for clinical disease during the study period.

The primary measure of interest of this study was rumen development. All areas of the rumen were sampled and measured as shown in Figure 1. Tables 1, 2, and 3 along with their accompanying Figures 2, 3, and 4, show the rumen papillae length, width, and density. These measurements are primary aspects of the rumen that are related to the tissue surface area available for volatile fatty acid absorption. Each area grows at a different rate and is quite different in size, even in adult ruminants. Therefore the sampling pattern as shown was used to quantify the entire reticulorumen.

Papillae length had five measured areas where the 6 g/hd/d treatment was significantly greater (P<0.10) than either the control (0 g/hd/d) or the 12 g/hd/d treatments. In 6 of 9 areas papillae width was significantly greater (P<0.05) in the 6 g/hd/d group than the control and in 4 areas was greater than the
Figure 1. Rumen sampling method: A = caudal portion of caudal ventral blind sac, RB and LB = right and left caudal dorsal blind sac, RC and LC = right and left cranial dorsal blind sac, RD and LD = right and left cranial ventral sac, RE and LE = right and left caudal ventral blind sac.

Table 1. Least squares means of papillae length (cm) for 9 areas of the rumen in calves fed 0, 6, or 12 g/d of amylase in grain.

<table>
<thead>
<tr>
<th>Amaize™ Comparison of means (g/head per day)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rumen area</td>
<td>0</td>
</tr>
<tr>
<td>RB</td>
<td>0.092</td>
</tr>
<tr>
<td>LB</td>
<td>0.104</td>
</tr>
<tr>
<td>RC</td>
<td>0.073</td>
</tr>
<tr>
<td>LC</td>
<td>0.101</td>
</tr>
<tr>
<td>RD</td>
<td>0.192</td>
</tr>
<tr>
<td>LD</td>
<td>0.139</td>
</tr>
<tr>
<td>RE</td>
<td>0.060</td>
</tr>
<tr>
<td>LE</td>
<td>0.041</td>
</tr>
<tr>
<td>A</td>
<td>0.099</td>
</tr>
</tbody>
</table>

1Rumen areas sampled: RB = right caudal dorsal blind sac, LB = left caudal dorsal blind sac, RC = right cranial dorsal blind sac, LC = left cranial dorsal blind sac, RD = right cranial ventral blind sac, LD = left cranial ventral blind sac, RE = right caudal ventral blind sac, LE = left caudal ventral blind sac, A = caudal portion of caudal ventral blind sac. Figure 1 shows location of these areas.

Table 2. Least squares means of papillae width (cm) for 9 areas of the rumen in calves fed 0, 6, or 12 g/d of amylase in grain.

<table>
<thead>
<tr>
<th>Amaize™ Comparison of means (g/head per day)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Rumen area</td>
<td>0</td>
</tr>
<tr>
<td>RB</td>
<td>0.057</td>
</tr>
<tr>
<td>LB</td>
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<tr>
<td>LC</td>
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<tr>
<td>RD</td>
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</tr>
<tr>
<td>LD</td>
<td>0.068</td>
</tr>
<tr>
<td>RE</td>
<td>0.051</td>
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<tr>
<td>LE</td>
<td>0.048</td>
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<tr>
<td>A</td>
<td>0.059</td>
</tr>
</tbody>
</table>

1Rumen areas sampled: RB = right caudal dorsal blind sac, LB = left caudal dorsal blind sac, RC = right cranial dorsal blind sac, LC = left cranial dorsal blind sac, RD = right cranial ventral blind sac, LD = left cranial ventral blind sac, RE = right caudal ventral blind sac, LE = left caudal ventral blind sac, A = caudal portion of caudal ventral blind sac. Figure 1 shows location of these areas.
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Table 3. Least squares means of papillae per cm² for 9 areas of the rumen in calves fed 0, 6, or 12 g/d of amylase in grain.

<table>
<thead>
<tr>
<th>Rumen area¹</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>SE</th>
<th>6 &gt; 0</th>
<th>12 &gt; 0</th>
<th>6 &gt; 12</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB</td>
<td>69.81</td>
<td>92.76</td>
<td>83.84</td>
<td>7.71</td>
<td>0.04</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>67.28</td>
<td>79.76</td>
<td>80.57</td>
<td>9.23</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>RC</td>
<td>53.80</td>
<td>74.99</td>
<td>66.73</td>
<td>7.42</td>
<td>0.05</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>LC</td>
<td>59.61</td>
<td>70.89</td>
<td>82.31</td>
<td>9.83</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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</tr>
<tr>
<td>RD</td>
<td>77.37</td>
<td>69.38</td>
<td>86.06</td>
<td>6.61</td>
<td>NS</td>
<td>NS</td>
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</tr>
<tr>
<td>LD</td>
<td>69.97</td>
<td>72.28</td>
<td>88.55</td>
<td>6.01</td>
<td>NS</td>
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<td>0.06</td>
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</tr>
<tr>
<td>RE</td>
<td>51.80</td>
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</tr>
<tr>
<td>LE</td>
<td>48.83</td>
<td>63.90</td>
<td>67.67</td>
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<tr>
<td>A</td>
<td>70.03</td>
<td>83.39</td>
<td>90.18</td>
<td>9.17</td>
<td>NS</td>
<td>NS</td>
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<td></td>
</tr>
</tbody>
</table>

¹Rumen areas sampled: RB = right caudal dorsal blind sac, LB = left caudal dorsal blind sac, RC = right cranial dorsal blind sac, LC = left cranial dorsal blind sac, RD = right cranial ventral blind sac, LD = left cranial ventral blind sac, RE = right caudal ventral blind sac, LE = left caudal ventral blind sac, A = caudal portion of caudal ventral blind sac. Figure 1 shows location of these areas.

Figure 2. Least squares means of papillae length (cm) for 9 areas of the rumen in calves fed 0, 6, or 12 g/d of Amaize™ in grain.

Figure 3. Least squares means of papillae width (cm) for 9 areas of the rumen in calves fed 0, 6, or 12 g/d of Amaize™ in grain.
12 g/hd/d group. In no cases was the 12 g/hd/d group greater than controls. In papillae per cm², there were three areas where the 6 gm/hd/d group were larger (P < 0.05) than the controls and two areas where the 12 g/hd/d were greater than controls along with two areas where the 12 g/hd/d group were greater than the 6 g/hd/d group. Rumen wall thickness was not affected by treatment. These data show that the amylase affected rumen growth and increased parameters related to nutrient absorption. The lack of difference in rumen wall thickness is not surprising as this is quite consistent across all areas of the rumen.

Grain intake was similar across all treatment groups and was quite typical of calves in summer temperatures and appeared adequate to support weaning of the calves by the end of the experiment. All animal growth measurements taken including heart girth, body weight, hip width, and withers height were similar for all treatments. These appeared normal for calves of this age and feeding regime with moderate levels of milk replacer and grain intake.

In summary, the enzyme systems of the neonatal calves may be able to utilize amylase and other enzymes to improve ruminal fermentation and nutrient absorption. Further research is needed to understand the optimal levels and types of enzymes that are beneficial for neonatal calves.

**Table 4. Least squares means of rumen wall thickness (cm) for 9 areas of the rumen in calves fed 0, 6, or 12 g/d of amylase in grain.**

<table>
<thead>
<tr>
<th>Rumen area</th>
<th>Amazie&lt;sup&gt;TM&lt;/sup&gt; (g/head per day)</th>
<th>Comparison of means</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>RB</td>
<td>0.126</td>
<td>0.141</td>
<td>0.134</td>
</tr>
<tr>
<td>LB</td>
<td>0.126</td>
<td>0.136</td>
<td>0.136</td>
</tr>
<tr>
<td>RC</td>
<td>0.138</td>
<td>0.150</td>
<td>0.144</td>
</tr>
<tr>
<td>LC</td>
<td>0.159</td>
<td>0.152</td>
<td>0.158</td>
</tr>
<tr>
<td>RD</td>
<td>0.176</td>
<td>0.175</td>
<td>0.157</td>
</tr>
<tr>
<td>LD</td>
<td>0.161</td>
<td>0.190</td>
<td>0.174</td>
</tr>
<tr>
<td>RE</td>
<td>0.140</td>
<td>0.139</td>
<td>0.131</td>
</tr>
<tr>
<td>LE</td>
<td>0.139</td>
<td>0.147</td>
<td>0.144</td>
</tr>
<tr>
<td>A</td>
<td>0.130</td>
<td>0.130</td>
<td>0.130</td>
</tr>
</tbody>
</table>

<sup>1</sup>Rumen areas sampled: RB = right caudal dorsal blind sac, LB = left caudal dorsal blind sac, RC = right cranial dorsal blind sac, LC = left cranial dorsal blind sac, RD = right cranial ventral blind sac, LD = left cranial ventral blind sac, RE = right caudal ventral blind sac, LE = left caudal ventral blind sac, A = caudal portion of caudal ventral blind sac. Figure 1 shows location of these areas.
dairy calf are quite complex, yet well suited for an animal that begins life as a monogastric and is transformed to a ruminant. The development of the rumen is paramount in importance from a modern-day management standpoint where economics and labor management are limiting factors on progressive dairy farms.

References


Le Huërou-Luron, I. 2002. Production and gene


Meeting the educational needs of dairy clientele in 2020

MICHAEL F. HUTJENS
Department of Animal Sciences, University of Illinois, Urbana, Illinois, USA

Changes in dairy production patterns

Ways in which the educational needs of the dairy clientele in 2020 and beyond are met will change compared to those used in historical and contemporary dairy educational programs. After interacting with dairy clientele (farm managers, agribusiness personnel, veterinarians, educators, and consultants) for over 33 years, new ‘teaching’ approaches and opportunities are appearing and will continue to develop in all sectors of the industry. Looking back to examine changing industry patterns allows us to look ahead and begin making strategic plans to serve this dynamic group. Nutrition and feed management examples and applications will be used in this paper, but these concepts could apply to reproduction, milk quality, genetics, manure management, and other related disciplines.

LOOKING BACK: CHANGES IN THE PAST 10 YEARS

The largest shift in US dairy production patterns has been where milk is being produced in the country. The western area in 1993 produced 35% of all US milk and had 31% of the dairy cows. In 2002, the West accounted for 41% of US dairy cows and produced 45% of all milk, growing in average herd size from 190 cows to 412 cows per herd. Table 1 summarizes changes over the last 10 years (1993 to 2003). Summary points are listed below.

- Total milk yield increased on average 1.2% annually. The trend toward more milk may shift, depending on the aging US population, new dairy products (e.g., a ‘new’ pizza), health concerns, advertising and marketing of dairy products, and export opportunities.
- Total cow numbers declined less than 1% annually. This trend may slow depending on trends in consumption (demand for dairy products).
- Milk yield per cow increased on average 2.1% annually, a figure which includes the impact of BST (commercial use began in 1994). Opportunities remain, as the average milk yield is 18,814 lbs while many herds average over 25,000 lbs and individual cows can exceed 40,000 lbs of milk. Larger herd sizes are associated with increased milk yield per cow.
- The number of dairy herds has dropped by 44%. This trend will continue as herd sizes increase to meet the demand for milk by consumers. The shift to larger herd size will reduce the number of dairy farm managers while increasing demands for unique services (feeding, reproduction, health, and financial consultants).
- Average herd size doubled in the last 10 years. This trend will depend on the management style of the farm manager or family.
- Milk prices remain constant, leading to tighter profits margins and increased emphasis on efficiency.

Table 1. US dairy statistics, 1993 and 2003.

<table>
<thead>
<tr>
<th></th>
<th>1993</th>
<th>2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total milk produced, billion lbs</td>
<td>151.0</td>
<td>169.8</td>
</tr>
<tr>
<td>Number of cows, million</td>
<td>9.705</td>
<td>9.025</td>
</tr>
<tr>
<td>Number of dairy farms</td>
<td>124,942</td>
<td>70,209</td>
</tr>
<tr>
<td>Herd size, cows per farm</td>
<td>78</td>
<td>130</td>
</tr>
<tr>
<td>Milk yield per cow, lbs per year</td>
<td>15,554</td>
<td>18,814</td>
</tr>
<tr>
<td>Milk price (Class 3), $ per 100 lb</td>
<td>11.80</td>
<td>11.40</td>
</tr>
</tbody>
</table>

Halladay, 2004
THE DAIRY INDUSTRY IN 2020

Milk production will increase while cow numbers decline and dairy farm numbers will drop. Larger herd sizes (250 to 3000 cows per unit with multiples of these base numbers) will continue. Dairy farms will produce 1.2 to 1.5 million lbs of milk per full time employee equivalent. Milk processing companies may target semi-load(s) of milk from the farm (600 milking cows per load). Table 2 summarizes projected numbers of dairy farms in the United States, assuming that milk consumption of dairy products remains constant (LaDue et al., 2004).

Table 2. Projected number of dairy farms in the US.

<table>
<thead>
<tr>
<th>Farm size (number of cows)</th>
<th>2000</th>
<th>2010</th>
<th>2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 49</td>
<td>52,920</td>
<td>18,235</td>
<td>2,821</td>
</tr>
<tr>
<td>50 to 99</td>
<td>31,360</td>
<td>12,751</td>
<td>3,549</td>
</tr>
<tr>
<td>100 to 199</td>
<td>12,865</td>
<td>7,204</td>
<td>2,655</td>
</tr>
<tr>
<td>200 to 499</td>
<td>5,350</td>
<td>4,292</td>
<td>2,335</td>
</tr>
<tr>
<td>Over 500 cows</td>
<td>2,675</td>
<td>4,292</td>
<td>3,361</td>
</tr>
<tr>
<td>Total</td>
<td>105,170</td>
<td>45,777</td>
<td>14,721</td>
</tr>
</tbody>
</table>

LaDue et al., 2004

Large herds (over 500 cows) increased cow numbers by 4% over the last year. The majority of milk will be produced on farms milking over 500 cows. Distribution of cows within herds will be a key factor that could impact sustainability of the operation and the need for various types of educational alternatives. Table 3 lists the distribution of milk production by herd size. Factors that could alter trends are outlined below.

- Marketing risk could be a factor if milk processors provide incentives for larger milk volumes picked up at the farm. The swine industry has future contracting and shackle space guarantees to ensure timely marketing. Will smaller dairy farms have a market?

Table 3. Percentage of milk production by herd size in the US.

<table>
<thead>
<tr>
<th>Farm size (number of cows)</th>
<th>2000</th>
<th>2010</th>
<th>2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 49</td>
<td>9.5</td>
<td>3.4</td>
<td>0.6</td>
</tr>
<tr>
<td>50 to 99</td>
<td>19.4</td>
<td>8.2</td>
<td>2.3</td>
</tr>
<tr>
<td>100 to 199</td>
<td>17.3</td>
<td>10.1</td>
<td>3.8</td>
</tr>
<tr>
<td>200 to 499</td>
<td>18.0</td>
<td>15.3</td>
<td>8.7</td>
</tr>
<tr>
<td>Over 500 cows</td>
<td>35.8</td>
<td>63.0</td>
<td>84.6</td>
</tr>
</tbody>
</table>

LaDue et al., 2004

Dairy managers as education clients

In the education process to date, the primary focus has been the dairy farm manager. Dairy farm systems can be divided into different management groups requiring alternative methods for education delivery.

FAMILY DAIRY SYSTEMS

Traditional ‘family’ dairy systems will continue to be a primary management group. This group will have the following characteristics that will dictate educational approaches.

- The labor source will include the owner, daughter(s) and son(s) in-law, herdsperson and/ or part-time youth.
- The size of the operation will depend on the number of family members employed with a target of 50 to 70 cows per full time employee equivalent.
- Milk production per cow will be high (25% above the national average).
- Sales of surplus dairy cattle can be a source of income. Registered dairy cattle breeders may remain in this category.

Educational programs that could appeal to this group include traditional approaches such as mass media, educational meetings, tours, and targeted topics (accelerated calf growth, lameness, milk components, and crossbreeding).
LOW INPUT DAIRY SYSTEMS

Low input dairy systems are popular in regions that have land suited to grazing and a traditional dairy history. These dairy farms have been referenced as ‘sustainable farms’ with the following characteristics.

- Family members are the only source of labor.
- Herd size will remain stable over time dictated by labor and land resources.
- Milk yield may be below the national average (90%).
- The ‘New Zealand system’ dairies are included in this group (pasture systems, larger number of cows (100 to 150 lactating cows per full time equivalent), and large parlors to reduce milking time to less than 2 hrs per milking).
- Investment per cow is minimized with little equipment owned, custom planting of crops and harvesting of forages, and/or off-farm purchase of feeds.
- Off-farm employment may be needed for supplemental income and insurance benefits.

The educational needs of this group will be specific for individual dairy managers and will apply to their particular situation. Tours, success stories in mass media, and production tips will be useful educational approaches. These individuals will need educational efforts to be close to their farms, as travel time or employment will be limiting factors. Weekend programs may be needed.

INTENSIVE DAIRY FARMS

Intensive dairy farms reflect larger dairy units (listed in Tables 2 and 3). These farms will increase in number as competition and milk processors favor these systems. The following characteristics can apply to this dairy model.

- Herd size will vary from 250 to 3000 cows with multiples of each size per farm (for example a herd of 12,000 cows may consist of units with 3000 cows for optimal management by one management team).
- Milk yield will be high as the latest technology and health programs will enhance performance and quality.
- Labor will be provided by outside resources, necessitating labor management skills and strategies for dealing with language limitations.
- Economics will be the driving factor as decisions are based on return on investment (not convenience or drudgery).
- The ability to assemble semi-loads of milk will allow market alternatives and premiums.
- Profit margins for suppliers will be tight as volume discounts may be expected by these dairy managers.

This group will require unique educational opportunities that may be targeted specifically for their farm(s), for example heat detection systems, feed delivery, or benchmarks to monitor economic success. The educational program may be delivered at the farm level allowing employees or managers to balance time availability with specific farm learning needs. Attending traditional educational programs is not popular; while networking with similar operations is perceived as more useful (invitational meetings, one-on-one sessions with specialists, and company-sponsored activities).

Agri-business as client

A second group of clients for educational programs are agri-business ‘educators’ as they serve dairy managers. ‘Training the trainer’ multiplies educator impact, resulting in more effective changes on farms, and a greater ability to deliver the recommendation (for example by including the educational concept in the feed delivered to the farm). Three groups are outlined below.

VETERINARIANS

Veterinarians are ‘the’ on-farm experts (defined by the dairy manager) that understand herd dynamics and health changes. If the veterinarian supports a feeding or management change, it will happen on her or his client farms. The veterinarian can be an effective method to indirectly reach dairy managers. If one Midwest dairy veterinarian is ‘sold’ on a concept (for example drenching cows), she or he can implement this program on 30 to 60 dairy farms. Training for veterinarians can include AABP (American Association of Bovine Practitioners).
Meeting the needs of dairy cattle clientele in 2020

national meetings and programs, state veterinary meetings, and local programs. Some states require continuing education (CE) credits be earned by veterinarians (another reason why veterinarians are potential clients).

REGIONAL FEED SUPPLIERS

Regional feed suppliers can react quicker than national feed suppliers. These suppliers are aware of local needs, are seen as part of the community (anti-Walmart image), and maintain ties to the community. These organizations may not have large research and marketing departments, and need to develop ways of staying current and updated.

CONSULTANTS

Consultants will play a more important role as increased herd sizes creates the demand for more specific information and guidelines. Many of these individuals will have advanced degrees (beyond the bachelor degree) and understand the science behind research studies. These individuals or groups will need to maintain cutting edge knowledge of their specialties through non-traditional approaches. Unique educational programs specific for this group (such as one-on-one discussions) may be popular.

The Illinois distance learning approach

Illinois Extension continues to change educational programs as their clientele base changes. With slightly over 1200 dairy farms (ranging from 27 to 2700 cows per herd), Illinois Extension delivers research results and recommendations via ten area Illinois Dairy Day programs, an annual dairy research summary, dairy records program using PC DART, and regional meetings arranged through the Four State Dairy Team (WI, MN, IA, and IL). Mass media plays a major role delivering information through Hoard's Dairyman, Dairy Today, Hoard's West, Dairy Herd Management, Midwest Dairy Business, AgriView, AgriNews and other media outlets. Participating in national and international programs expands the reach and focus of Illinois dairy extension programs. All information is available on the internet at http://www.traill.uiuc.edu/dairynet/.

Another opportunity occurred in 1998 when the University of Illinois offered instructional grants through a new program initiative called U of I On-Line. Administrators felt the future for education was on-line, reaching students across the state and around the world. Because dairy feeding classes had been taught across Illinois as traditional classes in the 1990s (classroom style with face-to-face lectures), a competitive grant was obtained ($122,200). The Illinois Dairy Certificate program was the only successful grant in the College of Agriculture and is outlined below.

- Five on-line classes were developed
  - Principles of Dairy Science (AnSci 200)
  - Advanced Dairy Management (AnSci 300)
  - Advanced Dairy Nutrition (AnSci 373)
  - Advanced Dairy Reproductive Physiology (AnSci 374)
  - Milk Secretion and Mastitis and Quality (AnSci 375)
- An on-line laboratory section is available to apply principles in the five classes including breed identification, CMT milk test, hand calculated and computerized ration formulation, feed identification, heifer growth, milk progesterone testing for pregnancy, and record analysis.
- When the student completes the five classes and lab section, they receive a Dairy Certificate from the University of Illinois (certified by the Graduate College at the University of Illinois). This is not a masters’ degree, but indicates successful completion of the course material and exams.

Individuals not interested in the Dairy Certificate Program can enroll in specific classes as needed or desired. The enrollment costs vary from $400 to $500 depending on whether the student is enrolled for credit (undergraduate or graduate) or audit (visitor) status and hours of credit. Each class has the following characteristics.

- Classes are conducted for 10 to 11 consecutive weeks.
- Each class has a CD that has all lectures recorded, edited, and ready to review when the student has time to listen during the week.
- Each week’s assignment consists of 4 to 8 modules with 15 minute listening time (single
topic lecture) including 15 to 20 slide sets that can be printed to take notes and list questions while listening.

- Once a week, instructor(s) will hold a formal class for 1 hr on the internet. Students can listen to the discussions, type in questions, and review homework. All 1 hr programs are recorded and archived for students that cannot attend or need to review.

- For students enrolled for academic credit, homework is assigned each week (requires 15 to 30 minutes to complete depending on the student’s background) and is sent electronically to the instructor for grading. A take-home final exam completes class assignments. Discussion questions and research paper reviews can be used by instructors to enhance the learning experience.

The CD can also be purchased as a reference tool to learn or use. The Advanced Dairy Feeding class (AnSci 373) has trained approximately 350 students using the internet class while over 1200 CDs have been sold ($45 for US students; $60 for foreign students). The CD can be purchased at http://www.fass.org/Uofi/.

Evaluation of the distance learning approach has been favorable to excellent (Hutjens and Baltz, 2000). The availability of dairy education on an ‘as needed’ basis, the flexibility of CD modules, ability to interact with instructors without being on campus, use of expertise from several different areas and universities, and CD teaching applications have been listed as advantages of the Illinois system.

Agri-business has also partnered with the Illinois CD/Internet distant learning approach through support of program development, distribution of the CDs to their key customers and participation in continuing education programs for specific disciplines.

### Future nutritional challenges

As Holstein herds achieve higher milk yields, nutrition needs increase to meet milk and reproduction requirements. The University of Illinois Extension conducted a national survey using the Monsanto Dairy Advisers and Technical Support Specialists. Each person was asked to rank in order five factors that they see limiting cows from reaching high yield milk. Thirty-one individuals responded including 17 veterinarians, six extension educators, and eight consultants. Table 4 summarizes their ranking and emphasis of the five factors.

<table>
<thead>
<tr>
<th>Item</th>
<th>Veterinarians</th>
<th>Extension agents</th>
<th>Consultants</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forage quality</td>
<td>13 (5)</td>
<td>5(3)</td>
<td>4(3)</td>
<td>33</td>
</tr>
<tr>
<td>Cow comfort</td>
<td>9</td>
<td>1</td>
<td>7(2)</td>
<td>24</td>
</tr>
<tr>
<td>Transition cow diets</td>
<td>8(5)</td>
<td>2</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>Feed bunk management</td>
<td>8(1)</td>
<td>3</td>
<td>4(1)</td>
<td>17</td>
</tr>
<tr>
<td>Feed availability</td>
<td>3(1)</td>
<td>2</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Feed consistency</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Feed particle length</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Feed refusal management</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Personnel mixing rations</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Dry matter intake</td>
<td>4 (3)</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Feed palatability</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Moldy feed</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Bunk silo management</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Water quality and management</td>
<td>2(2)</td>
<td>2(2)</td>
<td>4(1)</td>
<td>13</td>
</tr>
<tr>
<td>Nutrient balance and form</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>

The number in parentheses indicates the number of individuals that listed this factor as the number one ranked factor limiting cows for increased milk production. Each factor was ranked by awarding one point for the area or factor and an additional one point if it was the respondent’s first-ranked factor. Forage quality was number one in the group. Bunk management would have been first if all sub-factors (listed under bunk management in Table 4) were included as one factor. Respondents were specific about numerous feed bunk-related factors. It is also interesting to note the differences in the importance and variation of responses by job classification and on-farm responsibilities.

### In summary

After reviewing changes in the US dairy industry, clientele needs, and reduced extension commitments, the author’s biases on futuristic dairy educational programs are outlined below.

- The need of for research-based information will be remain important in 2020.
- Dairy managers will want more specific and targeted educational information that meets needs for their farms and employees.
• One-on-one training and education will be in greater demand (extension administration will not encourage this method of education delivery).

• Regional feed companies will be a factor if they can develop a local identity and meet local needs.

• The internet and distance learning approaches will continue to be a way to reach dairy clientele with information.

• Land grant universities will reduce faculty time for traditional extension programs, require cost recovery, and will emphasize the need for educational grants.

• The dairy industry (producers and agri-business) will need to come forward and support extension efforts (financially and vocally) or lose these educational efforts.

• Extension educators need to provide futuristic information (food safety, environment quality, consumer concerns, and systems approach) along with traditional information (feeding, mastitis, culling, and other management topics) in a format the clientele need and support.

References

Redefining selenium nutrition using organic selenium (Sel-Plex®): defining maximal acceptable tissue residues in beef

C.J. RICHARDS AND H.D. LOVEDAY

Animal Science Department, University of Tennessee, Knoxville, Tennessee, USA

Introduction

Selenium (Se) is an essential nutrient for humans and livestock. Beef cattle have a general requirement of 0.1 mg/kg of selenium in the diet (NRC, 1996). Many regions of the United States have soils that are low in Se. Because forage and feedstuff Se concentrations vary with soil Se concentrations, the Se status of US cattle herds is highly variable. Across the country 18.2% of the cows and heifers have blood Se concentrations that are considered low or marginal (Dargatz and Ross, 1996). In the southeastern region of the US, 42.4% of the cattle and 35.8% of the herds evaluated were considered to have at least a marginally deficient Se status. These deficiencies, along with stressors of marketing and transporting, results in many calves arriving at feedlots with varying degrees of Se reserves. As one of the major ingredients in feedlot cattle diets, corn contains a sufficient Se concentration (average = 0.13 mg Se/kg diet) to meet finishing cattle requirements. However, standard deviation on the 17 samples reported in the Nutrient Requirements of Beef Cattle (NRC, 1996) was 0.11 mg/kg. Because commodity feedstuffs are not commonly analyzed for microminerals, Se is routinely supplemented to ensure adequacy.

Since 1974, sodium selenate and selenite have been approved for use as supplements for livestock, with the feed industry primarily using the sodium selenite form. The two forms appear to have similar relative biopotencies (Mason and Weaver, 1986; Podoll et al., 1992), but in the functional ruminant, sodium selenite is known to be readily absorbed into feed particles or reduced to insoluble elemental Se or selenides in the acidic environment of the digestive tract (Podoll et al., 1992). This can result in most of the Se from selenite supplementation being excreted in feces (Lopez et al., 1969). This is of concern when supplementing beef cattle diets, because Se supplementation has been restricted to supplying a maximum of 0.3 mg/kg of Se by the US Food and Drug Administration (FDA, 1997). With the supplemental level limitations, it is desirable to have additional, more available sources of Se to use in beef cattle diets.

Selenium found in feedstuffs is most commonly associated with sulfur amino acids where it replaces the sulfur in methionine and cysteine (Levander, 1983). This is similar to the predominant form found in selenium yeast, which is selenomethionine (Kelly and Power, 1995). Research has shown that selenium from yeast is metabolized differently and has a greater availability than sodium selenite. Selenomethionine randomly substitutes for methionine in proteins while selenite is incorporated into selenoproteins (Finley, 2000).

Pehrson et al. (1999) and Gunter et al. (2003) have shown that feeding Se yeast from Sel-Plex® results in greater whole blood concentrations of Se than when sodium selenite is fed. However, data evaluating the effects of Se yeast supplementation on Se tissue concentrations in finishing cattle are not available. Therefore, the objective of this experiment was to determine the effect of feeding a basal diet balanced to meet the finishing cattle Se requirement or the basal diet supplemented with 0.3 mg/kg Se from Sel-Plex® Se yeast on corresponding calf performance, serum Se concentrations and to determine whether edible animal products (liver and muscle) exceed allowable Se concentrations.
Materials and methods

ANIMALS

Thirty-five heifers and 32 steers were purchased through local markets, vaccinated for IBR, BVD, PI3, and BRSV with Triangle 4 plus type II BVD (Fort Dodge, Overland Park, Kansas) and with a Clostridial 7-way plus Somnumune from AgriLabs (St. Joseph, MO). Calves were then delivered to The University of Tennessee Agricultural Experiment Station in Knoxville, Tennessee. Upon arrival, they were ear-tagged and tattooed in both ears, treated for internal and external parasites (EPRINEX®; Merial, Duluth, GA) and treated with Nuflor (Schering-Plough Animal Health, Union, NJ). Calves were maintained on a cool season pasture, and following a minimum interval of 28 days, all calves were weighed and heifers were verified open by ultrasonography. Twenty steers and 20 heifers were selected, blocked by sex and randomly allotted to one of eight groups of five calves. This provided four replicates per treatment (two replicates per sex). Groups were randomly assigned to pens. Pens were in a partially covered, concrete floor-feeding barn with free choice access to automatic water fountains. After calves were assigned to pens, they were fed a common diet (Table 1) containing 0.20 mg/kg supplemental Se from sodium selenite. Grain acclimation was accomplished by decreasing the levels of alfalfa pellets from 45% to 35%, 25%, and 15% with the difference being made up by cracked corn. Fish meal was included in the acclimation diets allowing calves to become accustomed to its consumption. High Se concentrations in fish meal allowed it to be an alternate feedstuff to be included in the final diet to maintain an adequate basal diet Se concentration if other feed ingredients varied between lots. Feed meal would replace a portion of corn and urea if needed. After the acclimation period, pens of calves were randomly assigned, within sex, to one of two finishing diet treatments. Treatment finishing diets consisted of a control basal diet (Table 2) balanced to contain 0.20 mg/kg (as-fed) of Se without supplemental Se and the basal diet plus 0.30 mg/kg (as-fed) of Se from Sel-Plex® (Alltech, Nicholasville, KY).

EXPERIMENTAL FINISHING PERIOD

This experiment was conducted as a double-blinded experiment in which only the researcher knew the composition of the Se premixes. Feeding personnel only knew the color code of Se premix to be included in each diet. Similarly, sample codes for lab analyses were labeled with an additional color-coding system so personnel conducting the Se assays did not know which samples corresponded to which dietary treatment code.

Table 1. Composition of initial grain acclimation diet.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% (Dry matter basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cracked corn</td>
<td>36.90</td>
</tr>
<tr>
<td>Alfalfa pellets</td>
<td>45.00</td>
</tr>
<tr>
<td>Corn gluten pellets</td>
<td>10.00</td>
</tr>
<tr>
<td>Molasses</td>
<td>5.00</td>
</tr>
<tr>
<td>Salt</td>
<td>0.30</td>
</tr>
<tr>
<td>Fish meal</td>
<td>1.80</td>
</tr>
<tr>
<td>Vit/mineral premixa</td>
<td>1.00</td>
</tr>
</tbody>
</table>

<CO-OP All Purpose Cattle Mineral, Tennessee Farmers Co-op, LaVergne, TN: Ca, 27.00%; P, 4.50%; Salt, 19.20%; Mg, 0.30%; K, 0.10%; Co, 24 mg/kg, Cu, 1,000 mg/kg, I, 36 mg/kg; Mn, 3,000 mg/kg; Se, 20 mg/kg; Zn, 3,500 mg/kg; Vit. A, 528,000 IU/kg; Vit. D, 99,000 IU/kg; Vit. E, 308 IU/kg.

Table 2. Composition of finishing diets.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% (Dry matter basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cracked corn</td>
<td>74.22</td>
</tr>
<tr>
<td>Alfalfa pellets</td>
<td>10.00</td>
</tr>
<tr>
<td>Corn gluten pellets</td>
<td>10.00</td>
</tr>
<tr>
<td>Molasses</td>
<td>3.50</td>
</tr>
<tr>
<td>Urea</td>
<td>0.38</td>
</tr>
<tr>
<td>Vitamin and mineral supplement a</td>
<td>1.75</td>
</tr>
<tr>
<td>Treatment premixa b</td>
<td>0.15</td>
</tr>
</tbody>
</table>

aSee Table 3 for composition of vitamin/mineral supplement.
bSee Table 4 for composition of selenium premixes.

Calf weights were recorded two consecutive days immediately prior to initiation of feeding experimental diets. Calves were weighed every 28 days for the duration of the experiment except for the final period, which was 18 days. Final weights were the average of weights taken the final two days.

Calves were fed their respective diets free choice, with daily replenishment. Prior to daily feeding, any feed present from the previous day’s feeding was removed from the feed bunk, weighed, recorded, and discarded. To verify Se concentrations of the finishing diets, a 0.45 kg sample was collected from each daily batch of complete finishing diet. These samples were collected, composited into a weekly sample, and a 0.91 kg subsample retained for Se analysis. Additionally, a 0.45 kg sample of all major feed
ingredients was collected upon delivery to the animal facility and Se content determined to verify a consistent concentration in the basal diet.

The control treatment premix contained only fine ground corn (Table 4). The Sel-Plex® treatment premix consisted of ground corn with 200 mg/kg of Se from Sel-Plex®. Treatment premixes were sampled and analyzed for Se content before feeding to ensure proper inclusion level. Both treatment premixes were blended in the treatment diets to provide 0 or 0.3 mg/kg of Se to yield total dietary concentration of 0.2 or 0.5 mg/kg Se. Both finishing diets used the same vitamin and mineral supplement, which did not contain supplemental Se, at a rate of 1.75% (dry matter basis) of the total diet. Final feeds were manufactured daily by mixing the major feed ingredients, supplement, and premixes in a horizontal paddle mixer (Marion Mixers, Inc.; Marion, IA) and then slowly adding liquid molasses.

Table 3. Composition of finishing diet vitamin/mineral supplement and mineral premix.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% (Dry matter basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin/mineral supplement</td>
<td></td>
</tr>
<tr>
<td>Limestone</td>
<td>80.00</td>
</tr>
<tr>
<td>Salt</td>
<td>17.14</td>
</tr>
<tr>
<td>Mineral premix</td>
<td>1.71</td>
</tr>
<tr>
<td>Vitamin premixa</td>
<td>1.14</td>
</tr>
<tr>
<td>Mineral premix</td>
<td></td>
</tr>
<tr>
<td>Fine ground corn</td>
<td>45.76</td>
</tr>
<tr>
<td>Molasses</td>
<td>4.00</td>
</tr>
<tr>
<td>Magnesium oxide</td>
<td>17.70</td>
</tr>
<tr>
<td>Zinc sulfate</td>
<td>16.55</td>
</tr>
<tr>
<td>Iron oxide</td>
<td>9.00</td>
</tr>
<tr>
<td>Manganous oxide</td>
<td>2.60</td>
</tr>
<tr>
<td>Copper sulfate</td>
<td>3.95</td>
</tr>
<tr>
<td>Potassium iodide</td>
<td>0.44</td>
</tr>
</tbody>
</table>

CO-OP Vitamin ADE Micro Mix, Tennessee Farmers Co-op, LaVergne, TN; Vitamin A, 11,000 IU/kg; Vitamin D, 880 IU/kg, and Vitamin E, 5.5 IU/kg.

Table 4. Composition of finishing diet treatment premixes.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control % (as-fed basis)</th>
<th>Sel-Plex® % (as-fed basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine ground corn</td>
<td>100.00</td>
<td>80.75</td>
</tr>
<tr>
<td>Sel-Plex®a</td>
<td>0.00</td>
<td>19.25</td>
</tr>
</tbody>
</table>

aAlltech, Nicholasville, KY.

Blood was collected from all calves on days 1, 28, 56, 84, 112, and 129. Whole blood was obtained via jugular venipuncture and collected into Vacutainers® (Becton Dickinson and Co., Franklin Lakes, NJ). Samples were immediately placed on ice, taken to the laboratory the same morning, allowed to coagulate, centrifuged for 20 min (2,600 x g), serum harvested, and frozen (-4°C) for subsequent Se analysis.

HARVEST MEASUREMENTS

On day 130, animals were loaded for overnight transport to a commercial harvest facility. Animals were harvested on day 131. Carcasses were identified, hot carcass weights recorded, and liver samples collected. Liver samples were collected from each liver lobe (approximately 100 g from each lobe for a total of 300 g). Samples were placed in labeled plastic bags and packed in dry ice for transport to the laboratory where they were stored frozen (-4 C) until analyzed. After a 48 hr chill, standard USDA Yield Grade and Quality Grade data were recorded as determined by a USDA grader. Adjusted fat thickness was determined by measuring fat depth ¾ the distance around the longissimus muscle at the 12th rib. This fat measurement was then subjectively adjusted according to USDA guidelines to reflect the overall carcass fatness. Ribeye area (sq cm) was measured using a standard USDA grid. Each 12th rib longissimus muscle area was measured twice and the average recorded. The percentage of kidney, pelvic and heart fat (% KPH) was determined to the nearest 0.5%. These yield grade factors were used in the following USDA Yield Grade formula: YG = 2.50 + (2.50 x adjusted fat thickness) + (0.20 x %KPH) + (0.0038 x hot carcass weight) – (0.32 x ribeye area). Carcass maturity and marbling degree evaluations were used to determine USDA Quality Grade. After completing the carcass evaluations, loin tissue samples (500 g) were collected at the 12th rib, placed in plastic bags, identified, and packed in dry ice for transport to the laboratory where they were stored frozen (-4°C) until analyzed.

SELENIUM DETERMINATION

Upon retrieval from the freezer and thawing, a scalpel blade was used to remove similar slices (by weight) from each of the three liver lobe sections of each animal to be combined for a single analytical observation. This procedure was repeated two more times to produce triplicate samples. Each loin sample was divided into four approximately equal quadrants and a core sample was obtained from each quadrant using a No. 10 cork borer (150 mm in diameter) so that a sample from each quadrant was represented in each sample for Se analysis. This procedure was
repeated two more times to produce triplicate samples. Sample size from this process was approximately 6 g. This was larger than could be digested in available test tubes, so loin samples were predigested and subsampled for Se determination. Feed samples were ground to pass a 1 mm screen in a Wiley Mill (Arthur H. Thomas Company, Philadelphia, PA) prior to analysis.

Selenium concentration of all samples (feed, serum, meat and liver) was determined using methodology based upon the fluorometric procedure by Koh and Benson (1983) as published by the American Association of Analytical Chemists (AOAC, 1990). Samples were analyzed for Se in triplicate and a coefficient of variation (CV) determined. If triplicates of a sample had a CV higher than previously determined acceptable, samples were rerun. In addition, a standard from the National Institute of Standards and Technology (No. 1577b bovine liver) of known Se content was assayed with each run of samples. If the bovine liver standard for a run was not within the acceptable range, the run was reanalyzed. All feed, serum and tissue Se concentrations are reported on an as-is basis.

STATISTICAL ANALYSIS

Feed consumption, growth performance, and tissue Se concentrations were analyzed as a randomized complete block design using the Mixed Procedure of SAS (Version 8.2, 1999; Cary, NC). The model included sex, diet treatment and the interaction with pen as the experimental unit. Differences in means were separated using the LSD procedure of SAS. Serum Se concentrations, collected at fixed times throughout the experiment, were analyzed using the repeated measure methodology of SAS (1999). For all response variables measured, results were considered significant at a confidence level of P<0.01.

Results and discussion

DIET SELENIUM

All feedstuffs were purchased from the local feed distributor as mill run ingredients without prior screening for Se content. Five lots of alfalfa pellets had an average Se concentration of 0.64 mg/kg and ranged from 0.47 to 0.94 mg/kg. Twelve lots of corn were all below the NRC (1996) published value of 0.13 mg/kg and averaged 0.08 mg/kg Se with values ranging from 0.05 to 0.11 mg/kg Se. Four lots of dry corn gluten feed pellets Se concentrations averaged 0.35 mg/kg and ranged from 0.35 to 0.38 mg/kg Se. One lot of fish meal contained 2.79 mg/kg Se. Grain acclimation diets were all supplemented with 0.2 mg/kg Se from a commercial vitamin/mineral premix (CO-OP All Purpose Cattle Mineral) and received an additional 0.06 mg/kg from fish meal; however, the diet Se concentrations varied due to changing alfalfa pellet:corn ratios. Analysis of feedstuffs used in the finishing treatment occurred after delivery to our research facility and before being incorporated into treatment diets. The control weekly diet Se analysis averaged 0.17 mg/kg Se with a standard deviation of 0.06 mg/kg and the Sel-Plex® diet averaged 0.49 mg/kg Se with a standard deviation of 0.04 mg/kg.

PERFORMANCE MEASURES

Initial body weights averaged 307 (±3.2 kg; Table 5) at allotment and averaged 360.8 (±3.5 kg) at the beginning of finishing diet treatments. Treatment had no effect on body weight on any of the weigh dates. A tendency (P>0.02) toward heifers being lighter than steers at the initiation of treatments became significant on day 56 due to steers weighing 41.4 kg more than heifers and remained significant at day 84. The sex effect was not significant (P>0.02) for weights taken on day 112 or the final weights and there was no treatment by sex interaction for any of the body weight measurements. Treatment had no effect on the average daily gain, feed intake, or gain:feed ratio for any of the four 28-day periods, the final 18 days, or the total treatment period. There were no significant sex effects or treatment by sex interactions for average daily gain, feed intake or gain:feed ratio.

Lack of change in growth performance, feed intake, and feed efficiency determined in this experiment were expected due to both diets exceeding the Se requirements of the cattle. The general recommendation for beef cattle dietary Se is 0.1 mg/kg (NRC, 1996) and the computer model, adjusted for conditions of this experiment, predicts a requirement of 1.48 mg/day. The average daily consumption of Se throughout the experiment was 2.85 times greater for the Sel-Plex® treatment with Controls consuming 1.71 mg/day and the Sel-Plex® group consuming 4.88 mg/hd/day of Se. No calves from either treatment showed signs or were treated
for illness while consuming experimental diets indicating that the addition of 0.3 mg/kg Se from Se yeast did not result in any negative health effects. These results agree with research from Hintze et al. (2002) in which steers were fed 55% concentrate diets that contained 0.62 or 11.9 mg/kg Se from feedstuffs. Other results from additional Se in deficient diets have been variable. When growing heifers, deemed to be receiving a Se deficient diet (Maas, 1998), were dosed with boluses releasing 3 mg of Se from sodium selenite each day, no performance differences were detected. In contrast, Nunn et al. (1996) determined that additional Se from Se boluses increased feed efficiency of steers offered a diet of Se deficient hay.

CARCASS MEASURES

No treatment differences were detected in hot carcass weight (average = 321.2 kg; Table 6), 12th rib fat (average = 1.41 cm), % KPH fat (average = 2.67), ribeye area (average = 83.25 sq cm), USDA yield grade (average = 3.0) or USDA quality grade (average = Choice®). No sex effect or treatment by sex interaction was detected for any measure. The lack of changes in carcass measures agrees with the lack of differences determined in performance.

SERUM SELENIUM

Concentrations of serum Se were affected by treatment, time and a treatment × time interaction (Figure 1). Serum Se was not affected by sex, a sex × time interaction or a treatment × time × sex interaction. On day one, there was no difference in serum Se concentration between the treatments. There was a difference between the Control and Sel-Plex® treatments at all subsequent measurements. Within the Control treatment, serum Se dropped from day 1 to 56 (0.091 to 0.078 µg/ml) and then reached a plateau (average = 0.080 µg/ml) with no point

Table 5. Weight gains, average daily gains, feed intakes and gain:feed ratios.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Treatment</th>
<th>P-value</th>
<th>SEM</th>
<th>Sex</th>
<th>Treatment</th>
<th>Treatment × Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allotment</td>
<td>304.0</td>
<td>309.9</td>
<td>3.2</td>
<td>0.02</td>
<td>0.26</td>
<td>0.97</td>
</tr>
<tr>
<td>Initiation</td>
<td>358.5</td>
<td>362.2</td>
<td>3.5</td>
<td>0.02</td>
<td>0.51</td>
<td>0.95</td>
</tr>
<tr>
<td>Day 28</td>
<td>402.9</td>
<td>401.0</td>
<td>7.8</td>
<td>0.03</td>
<td>0.87</td>
<td>0.37</td>
</tr>
<tr>
<td>Day 56</td>
<td>431.9</td>
<td>436.2</td>
<td>6.1</td>
<td>0.01</td>
<td>0.65</td>
<td>0.71</td>
</tr>
<tr>
<td>Day 84</td>
<td>477.4</td>
<td>478.2</td>
<td>6.1</td>
<td>0.01</td>
<td>0.94</td>
<td>0.97</td>
</tr>
<tr>
<td>Day 112</td>
<td>510.9</td>
<td>510.0</td>
<td>9.0</td>
<td>0.03</td>
<td>0.94</td>
<td>0.98</td>
</tr>
<tr>
<td>Final</td>
<td>524.9</td>
<td>523.0</td>
<td>8.2</td>
<td>0.02</td>
<td>0.88</td>
<td>0.81</td>
</tr>
<tr>
<td>Average daily gain, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>1.65</td>
<td>1.39</td>
<td>0.17</td>
<td>0.09</td>
<td>0.34</td>
<td>0.26</td>
</tr>
<tr>
<td>Period 2</td>
<td>1.04</td>
<td>1.26</td>
<td>0.20</td>
<td>0.49</td>
<td>0.48</td>
<td>0.39</td>
</tr>
<tr>
<td>Period 3</td>
<td>1.63</td>
<td>1.50</td>
<td>0.10</td>
<td>0.30</td>
<td>0.44</td>
<td>0.41</td>
</tr>
<tr>
<td>Period 4</td>
<td>1.20</td>
<td>1.14</td>
<td>0.17</td>
<td>0.34</td>
<td>0.81</td>
<td>0.99</td>
</tr>
<tr>
<td>Period 5</td>
<td>0.50</td>
<td>0.46</td>
<td>0.15</td>
<td>0.68</td>
<td>0.89</td>
<td>0.69</td>
</tr>
<tr>
<td>Overall</td>
<td>1.29</td>
<td>1.23</td>
<td>0.07</td>
<td>0.12</td>
<td>0.63</td>
<td>0.75</td>
</tr>
<tr>
<td>As-fed feed intake, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>9.3</td>
<td>9.4</td>
<td>0.2</td>
<td>0.02</td>
<td>0.61</td>
<td>0.73</td>
</tr>
<tr>
<td>Period 2</td>
<td>9.2</td>
<td>9.3</td>
<td>0.3</td>
<td>0.07</td>
<td>0.79</td>
<td>0.71</td>
</tr>
<tr>
<td>Period 3</td>
<td>10.3</td>
<td>10.3</td>
<td>0.5</td>
<td>0.57</td>
<td>0.97</td>
<td>0.81</td>
</tr>
<tr>
<td>Period 4</td>
<td>10.4</td>
<td>10.8</td>
<td>0.5</td>
<td>0.41</td>
<td>0.73</td>
<td>0.99</td>
</tr>
<tr>
<td>Period 5</td>
<td>10.0</td>
<td>9.9</td>
<td>0.4</td>
<td>0.21</td>
<td>0.82</td>
<td>0.54</td>
</tr>
<tr>
<td>Overall</td>
<td>9.8</td>
<td>9.9</td>
<td>0.3</td>
<td>0.16</td>
<td>0.83</td>
<td>0.94</td>
</tr>
<tr>
<td>Gain:feed ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>0.176</td>
<td>0.148</td>
<td>0.017</td>
<td>0.16</td>
<td>0.31</td>
<td>0.29</td>
</tr>
<tr>
<td>Period 2</td>
<td>0.113</td>
<td>0.135</td>
<td>0.020</td>
<td>0.75</td>
<td>0.49</td>
<td>0.35</td>
</tr>
<tr>
<td>Period 3</td>
<td>0.158</td>
<td>0.145</td>
<td>0.007</td>
<td>0.07</td>
<td>0.26</td>
<td>0.17</td>
</tr>
<tr>
<td>Period 4</td>
<td>0.113</td>
<td>0.105</td>
<td>0.011</td>
<td>0.37</td>
<td>0.60</td>
<td>0.95</td>
</tr>
<tr>
<td>Period 5</td>
<td>0.050</td>
<td>0.046</td>
<td>0.015</td>
<td>0.77</td>
<td>0.86</td>
<td>0.80</td>
</tr>
<tr>
<td>Overall</td>
<td>0.131</td>
<td>0.124</td>
<td>0.004</td>
<td>0.14</td>
<td>0.25</td>
<td>0.46</td>
</tr>
</tbody>
</table>

*Period 1 = days 1 to 28; Period 2 = days 29 to 56; Period 3 = days 57 to 84; Period 4 = days 85 to 112; Period 5 = days 113 to 130; Overall = days 1 to 130.
differing from the previous time point for the remainder of the experiment. This drop is associated with the change in dietary concentrations. Grain acclimation diets all had 0.2 mg/kg of supplemental Se in addition to varying levels from feedstuffs while the Control treatment had a total concentration of 0.2 mg/kg Se. In the Sel-Plex® treatment, serum Se increased between day 1 and 28 and then reached a plateau at an average concentration of 0.11 µg/ml. While this was 1.39 times the concentration in the Control, the design of this experiment did not allow differentiation between effects caused by increasing dietary Se concentrations in general or specific effects from Se yeast.

Hintze et al. (2002) conducted an experiment with growing beef cattle diets to determine if cattle from areas with naturally high Se feedstuffs could be viable sources of Se for human diets. They obtained steer calves from areas with moderate and high Se soil concentrations and consequently feedstuffs consumed by cattle. Those steers were fed diets containing 0.62 or 11.9 mg/kg Se. Steers from the high Se area had an initial plasma Se concentration of 0.56 µg/ml and steers from the moderate area had concentrations of 0.12 µg/ml. The serum levels from the moderate area are comparable to calves at initiation of the present experiment, but levels from the high Se areas are greater than any levels measured in the current experiment. Neither steers from the high Se area fed the 11.9 mg/kg diet or steers from the moderate area fed the 0.62 mg/kg diet showed a change in plasma Se concentrations. Steers from the moderate area fed

Table 6. Carcass weight, 12th rib fat, KPH fat, ribeye area, yield grade and quality grade.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Treatment</th>
<th>P-value</th>
<th>SEM</th>
<th>Sex</th>
<th>Treatment</th>
<th>Treatment x Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot carcass wt, kg</td>
<td>Control</td>
<td>323.2</td>
<td>7.0</td>
<td>0.13</td>
<td>0.71</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Sel-Plex®</td>
<td>319.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12th Rib fat, cm</td>
<td>Control</td>
<td>1.47</td>
<td>0.05</td>
<td>0.02</td>
<td>0.18</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Sel-Plex®</td>
<td>1.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KPH, %</td>
<td>Control</td>
<td>2.68</td>
<td>0.06</td>
<td>0.05</td>
<td>0.78</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>Sel-Plex®</td>
<td>2.65</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REA, sq cm</td>
<td>Control</td>
<td>85.0</td>
<td>3.6</td>
<td>0.56</td>
<td>0.53</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>Sel-Plex®</td>
<td>81.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yield grade</td>
<td>Control</td>
<td>3.0</td>
<td>0.2</td>
<td>0.29</td>
<td>0.98</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>Sel-Plex®</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quality gradec</td>
<td>Control</td>
<td>19.9</td>
<td>0.3</td>
<td>0.28</td>
<td>0.60</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>Sel-Plex®</td>
<td>20.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Kidney, pelvic and heart fat.
*REA = ribeye area.
*19 = Choice−, 20 = Choice0 and 21 = Choice+.

![Figure 1. Serum selenium concentrations.](image)

*Treatment within time, P>0.01
+Within treatment from previous time
the 11.9 mg/kg diet had increased plasma Se concentrations within two months to levels similar to steers previously exposed to high Se. Steers previously exposed to high Se levels and placed on the 0.62 mg/kg diet had plasma Se levels drop to concentrations that, at the end of the 105-day feeding period, were not different from calves originating from the moderate area and receiving the same diet. The lowest level of Se fed by Hintze et al. (2002) was slightly higher than the levels fed in our Sel-Plex® treatment and higher than our Control. Calves fed the 0.62 mg/kg diet in the experiment of Hintze et al. (2002) had similar plasma Se concentrations to serum concentrations in our Sel-Plex® calves (0.13 µg/ml vs 0.11 µg/ml, respectively). Both values are in a range described as normal (~0.10 µg/ml; Maas, 1998). Awadeh et al. (1998) found similar results when growing calves were fed diets containing 0.41 mg/kg (basal) and basal + sodium selenite to 0.73 mg/kg Se and showed that serum Se increased with dietary Se supplementation (0.12 µg/ml vs 0.29 µg/ml, respectively). Results from the above experiments, along with the changes during the initial points of the current experiment demonstrate serum Se concentrations closely following dietary levels of Se.

MEAT SELENIUM

Average meat Se concentration was 0.12 mg/kg higher for the Sel-Plex® treatment than Control (Figure 2) but was not affected by sex or a treatment by sex interaction. The Se content of the loin muscle, as collected in this experiment, has been determined to be representative of several of the edible muscle tissues including cuts from the round, sirloin, shoulder clod and ribeye muscles (Hintze et al., 2002).

While there was a 1.9-fold increase in the muscle Se concentration between the Control and Sel-Plex® treatments (Figure 2), both values are still lower than values obtained from calves in moderate or high Se areas of the United States and those fed diets consisting of feedstuffs raised in high Se regions. They were also lower than sheep fed a corn-based diet with the addition 0.3 mg/kg inorganic Se (Podoll et al., 1992) or the upper level determined normal by the US Food and Drug Administration (FDA, 1997; 0.4 mg/kg). In the experiment by Hintze et al. (2002), steers purchased from high Se regions arrived with muscle biopsy Se concentrations of 2.10 mg/kg, while calves from moderate regions averaged 0.40 mg/kg. After those calves were fed diets containing 11.9 or 0.62 mg/kg of Se, steers from the high region and fed the 11.9 mg/kg diet had ribeye muscle Se concentrations of 2.06 mg/kg and the moderate area with 0.62 mg/kg diet contained 0.35 mg/kg. While analyzing ground beef from animals raised in various regions of the US, Finley et al. (1996) found that Se concentrations varied from 0.34 mg/kg in samples obtained from cattle raised in eastern North Dakota to 0.06 mg/kg for samples from cattle raised in

![Figure 2. Meat selenium concentrations.](image-url)
southwestern Missouri. These experiments show that there is currently great variability in the Se content of beef reaching the retail market.

Hintze et al. (2002) suggested based on their data that 2.1 to 2.5 mg/kg could be the maximum amount of Se that can be incorporated into muscle. This would be consistent with findings that Se present in proteinaceous tissue is primarily selenomethionine and selenocysteine with the ratio of these forms depending on the feedstuffs consumed (Beilstein and Whanger, 1988). Because methionine and cysteine contents of muscle are not affected by Se addition to the diet, Se content of muscle could only increase to a relative level based on the proportions of methionine and cysteine containing Se. Therefore, differences in the incorporation rates of Se into muscle tissues due to form (inorganic vs organic) of dietary Se would be expected. Several publications have reported muscle incorporation comparisons between feeding of inorganic and organic Se sources and shown that Se from organic sources is more readily incorporated into tissues than inorganic sources of Se (Ullrey et al., 1977; Ammerman et al., 1980; Beilstein and Whanger, 1988; Ekholm et al., 1991). Despite incorporation rate differences between dietary forms, Hintze et al. (2002) noted there is high correlation between dietary and meat Se concentrations.

LIVER SELENIUM

Liver Se concentrations were affected by treatment, sex and a treatment × sex interaction (Figure 3). Average liver Se concentrations for cattle on the Sel-Plex® treatment were 0.41 mg/kg higher than that of the Control treatment. Liver concentrations of Se are generally related to dietary Se concentration, but not as highly correlated as other tissues (Hintze et al., 2001). Podoll et al. (1992) reported that sheep fed a high corn diet with 0.3 mg/kg Se supplementation from selenate or selenite had similar liver concentrations (1.79 and 2.10 mg/kg, respectively). Steers in the experiment of Hintze et al. (2002) had liver Se concentrations ranging from 0.89 to 5.94 mg/kg. Both of these experiments consisted of diets meeting the 0.3 mg/kg supplemental Se restriction, but have liver concentrations greater than the range of 0.1 to 1.2 mg/kg determined as normal by the FDA or those observed in this experiment.

The sex effect was a result of heifers within each treatment having higher liver Se concentrations than steers. The authors know of no other data that demonstrate an effect of sex on liver Se. While differences between sexes on the Control diet were small (0.02 mg/kg), heifers on the Sel-Plex® treatment had higher liver Se concentrations (0.19 mg/kg) than did steers on the same treatment, which led to the treatment × sex interaction. Because calves for this experiment were purchased through local market channels and most likely represent many sources and varied backgrounds, liver differences that occurred could be a carryover effect from prior management. Serum Se concentrations were not different at the initiation of the experiment, but they are noted to be more representative of Se status over a shorter period than liver tissues (Podoll et al., 1992).
Differences in responses of liver tissues to supplementation may be due to an adaptation to prolonged high dietary Se concentrations. Hintze et al. (2002) determined that calves from a moderate Se background and fed a high Se diet had higher liver Se concentrations than steers from a high Se background and fed high Se diets. They proposed that this resulted from differences in the up-regulation of Se methylation by S-adenosylmethionine, which is necessary for Se excretion. Another potential explanation, demonstrated in the rat liver, may be that 83% of Se is associated with GSH-Px compared to relatively low levels of that form in muscle (Behne and Wolters, 1983).

Implications

Results of this experiment indicate that supplementing 0.30 mg/kg of selenium from selenium yeast in a feedlot diet adequate in selenium does not affect animal performance, but does increase serum and tissue selenium concentrations. However, the range of normal liver concentrations provided by the Food and Drug Administration is 0.1 to 1.2 mg/kg of selenium. Selenium in meat is noted to be one-third to one-fourth the levels in the liver. This results in normal meat Se levels ranging from 0.025 to 0.4 mg/kg. Calves fed Sel-Plex® selenium yeast at 0.3 mg/kg had average liver selenium concentrations of 0.83 mg/kg and meat samples averaged 0.29 mg/kg. Therefore, selenium residues in edible tissue from feedlot beef cattle supplemented with 0.3 mg/kg selenium from selenium yeast do not exceed allowable levels or other published levels.

Acknowledgements

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Rumen acidosis: modeling ruminant response to yeast culture

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Introduction

Ruminal acidosis is a fairly well known digestive disorder (Owens et al., 1998). Acute acidosis occurs when a ruminant animal ingests quickly a large quantity of rapidly fermentable carbohydrates. As a consequence, the rumen pH decreases below 5.0 and lactic acid accumulates in the rumen fluid and in the blood. Death is a common outcome. Before this dramatic event, there is subclinical acidosis (5.5<pH<6.2), which is a frequent situation for high yielding animals receiving diets deficient in fibre and rich in highly digestible substrates formulated to meet high energy requirements. Several drawbacks are associated with subclinical acidosis. Low pH in the rumen over a long period of time inhibits intake and cell wall digestion. This last aspect alters the energy value of the diet, particularly of its forage component. Moreover, the VFA profile in the rumen fluid is altered with a low acetate: propionate ratio and sometimes a significant accumulation of lactic acid. One of the outcomes of subclinical acidosis is low milk fat content, which can fall below 3%. Several other diseases are associated with subclinical acidosis, as it is a contributing factor in abomasal displacement, liver abscesses and lameness.

The objective of this paper is an overview of the quantitative relationships that link aspects of rumen acidosis and use of yeast supplementation in ruminant diets, particularly diets fed lactating cows.

Methods

To reach this target we have considered the problem from the viewpoint of statistical modeling. Databases were constructed from experiments involving dietary yeast supplements published in scientific papers. Two databases were built from in vitro experimental data measured with either mono- or mixed cultures of rumen microbes. Two in vivo databases were also built. The first contained data on rumen fermentation and digestion measured in vivo on ruminally- and dual- (rumen and duodenal) cannulated animals. The second database contained trials performed on lactating dairy cows with simultaneous data of digestibility.

Interpretation of these databases was based on a statistical meta-analyses (St Pierre, 2001). The publications were separated into experiments that were individually encoded. The basic statistical model applied to the data was:

\[ Y_{ijk} = \mu + YEAST_{i} + EXP_{j} + E_{ijk} \]

Where:
- \( Y_{ijk} \): observations
- \( \mu \): overall mean
- \( YEAST_{i} \): effect of yeast (yeasts vs control)
- \( EXP_{j} \): influence of experiment j
- \( E_{ijk} \): residual error

All the models were used without weighting the observations.

Results

IN VITRO EXPERIMENTS

Monoculture and co-culture

A limited number of publications described
experiments where monocultures of rumen bacteria were performed with or without *Saccharomyces cerevisiae*. An initial goal was to study the impact of yeast supplements in the media on lactate utilisation by lactate fermenters, either *Megasphaera elsdenii* or *Selenomonas ruminantium* (Chaucheyras et al., 1995; Rossi et al., 1995; Callaway and Martin, 1997). There was a clear dose-dependent increase in the utilisation of lactate, suggesting a role for yeast in decreasing rumen acidosis. Unfortunately, as the measured items and the experimental conditions were very different it was difficult to empirically pool the data on lactate metabolism. Simultaneously the acetate:propionate ratio decreased as is shown in Figure 1.

Interesting experiments were also performed with cocultures of *M. elsdenii* and *Streptococcus bovis*, a lactate producer (Chaucheyras et al., 1995). In this work, adding yeast boosted the biochemical activity of the organism. Other trials demonstrated that yeast addition to the medium improved cellobiose digestion by fibrolytic bacteria, either *Fibrobacter succinogenes* or *Ruminococcus albus* (Callaway and Martin, 1997). Several assumptions were made to explain the mode of action of the yeast supplements, including provision of soluble growth factors (amino acids, organic acids, vitamins), or possibly by improving the anaerobic conditions.

One of the major difficulties in pooling and interpreting databases of mono- and co-cultures is identifying and controlling the specific experimental influences. This is particularly true when the number of available and (or) complete publications is limited, as is presently the case. A useful way to integrate these data is to create simple mechanistic models of *in vitro* fermentations to connect these experiments and to improve our comprehension of the mode of action of yeast. As an example, Figure 2 is a diagram of a model suited to test the influence of adding yeast on the lactate producers and lactate utilizers. With this model it was possible to achieve fairly good accuracy between simulated and the experimental data of Rossi et al. (1995).

**Mixed rumen mixed cultures**

A database was built from publications dealing with the impact of yeast on *in vitro* mixed cultures in rumen fluid. It contained 21 publications, pooling 49 experiments and 121 treatments. In each experiment there was a control compared to one or several treatments where a yeast supplement was added to the culture (n = 89) or to the donor’s rumen fluid (n = 32). There were two *in vitro* methods, either in batch (n = 69) or in continuous culture devices (n = 52). For each statistical treatment the provided information were the LS means (yeast vs control), the numbers of treatments (n) and of experiments (Nexp), the value of the residual standard deviation (rsd) and its unit, the probability of the test (P) and

---

**Figure 1.** Influence of adding yeast on the acetate:propionate ratio *in vitro* in co-culture with *M. elsdenii*.  

- Nisbet and Martin (1991)  
- Rossi et al. (1995)  
- Chaucheyras et al. (1995)  
- Calaway and Martin, 1997
the percentage of ‘aberrant treatments’ (a) which presented normalised residuals larger than 2. There was an increase in pH in response to yeast supplements (6.35 vs 6.32, n = 94, N exp = 37, rsd = 0.05, P = 0.028, a = 6.4%). Where data were few, this impact was partly confirmed as a trend (n = 82, P = 0.11) when the analysis was conducted within the experiment and with the volatile fatty acid (VFA) concentration used as a covariate. This trend suggested an eventual favorable effect of yeast on the pH of the medium. To go further in the analysis several sub-bases were built to respond to specific issues. When only the data with pH<5.5 were considered (Jouany et al., 1998; Lynch and Martin, 2002), the influence of yeast supplementation was more marked (pH increase was 0.055 vs 0.024) but less significant (5.32 vs 5.26, n = 14, N exp = 6, rsd = 0.05, P = 0.064, a = 7.1%). Pooling the data of Carro et al. (1992), Zelenak et al. (1994) and Lynch et al. (2002) allowed testing where there was any interaction between pH response and the level of cell wall (CW) in the diet or feed. The pH actually increased for feeds or diets having a higher level of CW, however there was no effect of yeast on pH and no interaction between CW and yeast.

There was no effect of treatment on VFA concentrations or production (66.38 vs 65.85, n = 95, N exp = 36, rsd = 5.0 mM, P = 0.62, a = 5.3%). The acetate:propionate molar ratio was slightly decreased, but this effect was not significant (2.99 vs 3.05, n = 98, N exp = 38, rsd = 0.20, P = 0.131, a = 5.1%). The proportions of the isoacids were not altered by yeast supplementation (6.33 vs 6.06, n = 38, N exp = 15, rsd = 0.36%, P = 0.31, a = 9.5%). Also, there appeared to be no influence of yeast supplementation on lactic acid concentration in the medium (0.646 vs 0.667, n = 32, N exp = 11, rsd = 0.105 mM, P = 0.603, a = 3.1%). Similarly, there was no effect when lactic acid concentration was corrected by the VFA concentration, the two items being linked.

The molecular hydrogen status did not seem to be altered by yeast. Effectively CH4 content or production (13.6 vs 13.4, n = 58, N exp = 23, rsd = 1.3 mM CH4, P = 0.576, a = 1.7%) and H2 content or production (0.444 vs 0.440, n = 44, N exp = 17, rsd = 0.060 mM H2, P = 0.842, a = 4.6%) were not significantly modified by adding yeast. For CH4 there was a positive correlation with VFA, however there was no effect of yeast when VFA were considered as
a covariate. Globally there was no influence of yeast on NH\textsubscript{3} content or production (154.4 vs 151.0, n = 55, N\textsubscript{exp} = 21, rsd = 10.6 mg N-NH\textsubscript{3}/L, P = 0.275, a = 7.3%). However, as the experiments measuring NH\textsubscript{3} levels lower than 100 mg N-NH\textsubscript{3}/L were selected, there was a significant increase in response to yeast (72.1 vs 57.8, n = 21, N\textsubscript{exp} = 7, rsd = 5.9 mg N-NH\textsubscript{3}/L, P = 0.002, a = 0%).

Microbial N production tended to be increased by adding yeast (837.4 vs 791.0, n = 17, N\textsubscript{exp} = 8, rsd = 52.1 mg microbial N/d, P = 0.107, a = 11.7%). Also the efficiency of microbial growth tended to be increased by yeast (21.8 vs 20.4, n = 17, N\textsubscript{exp} = 8, rsd = 1.48 g microbial N/kg RFOM, P = 0.099, a = 11.8%). The number of protozoa in the medium was unaffected by yeast (4.48 vs 4.53, n = 32, N\textsubscript{exp} = 13, rsd = 0.16 log\textsubscript{10} protozoa/ml, P = 0.494, a = 6.25%).

In contrast, the total number of viable bacteria was significantly increased by yeast (8.67 vs 8.57, n = 26, N\textsubscript{exp} = 9, rsd = 0.08 log\textsubscript{10} bacteria/ml, P = 0.009, a = 0%). Moreover, on a larger number of data, the number of cellulytic bacteria was significantly increased by yeast supplementation (7.65 vs 7.20, n = 32, N\textsubscript{exp} = 12, rsd = 0.32 log\textsubscript{10} bacteria/ml, P = 0.002, a = 6.2%). All these data suggested an increase of microbial proliferation by adding yeast in vitro.

The apparent degradability of substrate dry matter was not significantly increased by yeast addition (58.1 vs 57.7, n = 57, N\textsubscript{exp} = 22, rsd = 2.7%, P = 0.613, a = 7.0%). In contrast, on a smaller set of data the degradability of NDF was improved by yeast supplementation (53.3 vs 50.3, n = 32, N\textsubscript{exp} = 15, rsd = 4.2%, P = 0.062, a = 6.25%). This last aspect is consistent with the above cited results on the number of bacteria, particularly the cellulytic species.

**IN VIVO EXPERIMENTS**

**Rumen fermentation and digestion**

In order to quantify the effects of yeast on in vivo ruminal fermentation we pooled into a database the results of 55 publications corresponding to 78 experiments and 186 treatments. This database was much larger than the in vitro database. Yeast cultures were from at least eight different commercial preparations. Half of the trials (28 of 56) used Yeast Saccharomyces cerevisiae as a source of strain 1026. In some papers, two yeasts were compared. In all the papers, some rumen parameters were available, at least pH and/or VFAs. Some additional data were noted when available such as in vivo dry matter (OMD) digestibility, and in situ measurements. There was a lack of basic information on either yeast concentration, or live weight of the animals, or dry matter intake, or chemical analysis of the diet in some of the studies. The yeast effect was tested with the basic model including the trial and yeast effects.

There were non-significant increases in response to yeast supplements in pH (6.341 vs 6.320, n = 168, N\textsubscript{exp} = 70, rsd = 0.015, P = 0.288, a = 8.3%), in VFA concentrations (99.1 vs 97.8, n = 156, N\textsubscript{exp} = 64, rsd = 75.8 mM, P = 0.386, a = 8.8%), in the acetate/propionate ratio (3.24 vs 3.17, n = 163, N\textsubscript{exp} = 69, rsd = 0.078, P = 0.110, a = 3.7%), and a non-significant decrease in lactic acid concentration (1.388 vs 1.45, n = 39, N\textsubscript{exp} = 15, rsd = 0.417, P = 0.729, a = 10.3%). Except for the pH and the acetate/propionate ratio, these data were fairly similar to the in vitro responses.

Yeast tended to increase organic matter digestibility (71.4 vs 70.8%, n = 66, N\textsubscript{exp} = 31, rsd = 3.23, P = 0.148, a = 9.0%). There was a similar trend for increased in situ DM degradability (55.9 vs 55.0%, n = 75, N\textsubscript{exp} = 32, rsd = 7.12, P = 0.145, a = 5.3%), which was interestingly related with rumen pH within (w) the experiment (isDMDw = 10.0 pHw, n = 72, R\textsuperscript{2} = 26%, rsd = 1.8%, a = 4.0%). Otherwise it must be stressed that when the 23 treatments having less than 40% dietary concentrate were considered, there was a good fit and a significant increase of OMD (64.6 vs 63.2%, n = 23, N\textsubscript{exp} = 11, rsd = 1.33, P = 0.033, a = 0%). There was no statistical influence of yeast on whole tract digestibility of NDF (53.5 vs 54.0%, n = 47, N\textsubscript{exp} = 22, rsd = 2.19, P = 0.522, a = 8.5%) and ADF (48.4 vs 47.2%, n = 37, N\textsubscript{exp} = 16, rsd = 5.7, P = 0.555, a = 5.4%). This last aspect did not confirm the in vitro data.

When the yeast concentration was available in the paper or could be estimated because the yeast description was sufficient, we used a model including the trial effect and the yeast dose. It was expressed as log\textsubscript{10}(CFU/100 kg of live weight) because the database concerned cattle and sheep. Globally, similar conclusions could be obtained with this second approach. Nevertheless, it should be stressed that for pH, yeast dose is at the limit of the level of significance:

\[ \text{pH} = 6.32 + 0.00556 \log_{10} \text{(yeast concentration)} \]

(n = 147, N\textsubscript{exp} = 64, rsd = 0.032, P = 0.086, a = 8.2%)
This increase in pH value in response to yeast supplementation is consistent with the in vitro data.

In order to test whether some strains are more effective than others, we also studied two sub-databases concerning the two most studied strains: Yea-Sacc\(^{10268}\) and Diamond V. There was no statistical effect of the yeast whatever the sub-database concerned. In the residual of the negative equation linking pH and VFA concentration including a trial effect, the yeast effect was statistically significant for the whole database (P = 0.024) and for the Yea-Sacc\(^{10268}\) database (P = 0.034).

To look for any optimum yeast dosage, a dose effect has been tested on the 10 experiments where three levels were given. There was no dose effect on rumen pH, rumen volatile fatty acids, acetate:propionate ratio or ammonia concentration.

**Responses of lactating cows**

The database was constituted from 35 publications pooling 40 experiments and 122 treatments (3272 cows) to quantify the effects of yeast culture on dry matter intake (DMI), raw milk yield (RMY), milk fat content, milk protein content, and body weight change (BWC) of dairy cows. The choice of the data was based on the fact that industrial fermentation processes have rapidly changed since the first publications, consequently only the recent data were considered. The yeast were partitioned among different products (Diamond V-XP: 16 groups; Yeast+13 groups, Yea-Sacc\(^{10268}\): 16 groups, others: 21 groups (Cell-Con, Western 2x225, Levucell), which were pooled because of a low number of groups). Doses used largely differed according to the type of products. Diets were representative of most of the rations used in early- and mid-lactation with high concentrate diets for high producing cows. Most diets were fed as TMRs, once or twice a day. Because literature suggested that effects can differ according to stage of lactation, we coded this as a factor with three levels: EL for experiments starting from calving or during pregnancy and ending before 70 DIM (peak), ELML for experiments starting before peak and ending during mid-lactation, and ML for experiments starting past peak of lactation.

Data recorded for animal response to yeast supplement were dry matter intake (DMI), raw milk yield, milk fat content (MFC), milk protein content, and body weight change (BWC). Simultaneously, data on NDF, ADF and CP digestibility were recorded.

The basic model was applied and three others were also used:

\[
Y_{ijk} = \mu + YEAST + EXP + E_{ijk}
\]

to test the effect of each type of yeast.

\[
Y_{ijk} = \mu + YEAST + stage of lactation + YEAST \times stage of lactation + E_{ijk}
\]

to test the effect of yeast in interaction with stage of lactation (EL, ELML, or ML).

\[
Y_{ijk} = \mu + YEAST + %Conc + YEAST \times Conc + E_{ijk},
\]

to test the interaction between yeast and %Conc, where %Conc is the percentage of concentrate in the diet.

There was a trend (P = 0.08) toward increased raw milk yield by 1.3 kg when all types of yeast and all stages of lactation were taken together (Table 1). No effects of on milk composition, DMI or BW change were observed. ADF digestibility tended to be increased (P=0.15, +2.8%). This last result was consistent with data observed in vitro.

<table>
<thead>
<tr>
<th>No. of experiments</th>
<th>Ntreat</th>
<th>CTRL</th>
<th>Yeast</th>
<th>P &lt;</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter intake, kg/d</td>
<td>34</td>
<td>99</td>
<td>20.0 ± 0.3</td>
<td>20.2 ± 0.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Raw milk yield, kg/d</td>
<td>39</td>
<td>112</td>
<td>32.2 ± 0.4</td>
<td>31.3 ± 0.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Milk fat content, g/l</td>
<td>37</td>
<td>104</td>
<td>37.0 ± 0.2</td>
<td>37.1 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Milk protein content, g/l</td>
<td>37</td>
<td>104</td>
<td>31.5 ± 0.1</td>
<td>31.4 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body weight change, kg/d</td>
<td>34</td>
<td>34</td>
<td>-0.1 ± 0.4</td>
<td>0.1 ± 0.4</td>
<td>P=0.82</td>
</tr>
<tr>
<td>N digestibility, %</td>
<td>6</td>
<td>15</td>
<td>67.4 ± 1.7</td>
<td>69.9 ± 1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADF digestibility, %</td>
<td>6</td>
<td>13</td>
<td>46.6 ± 1.6</td>
<td>49.4 ± 1.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^{1}\) Data are presented as least square means ± se. RMSE: root mean square error of the model.
When concentrate percentage was included in the analysis, no effect of yeast and no interaction between yeast and percentage of concentrate was significant (data not shown). The role of some other possible interfering factors were also tested. As for concentrate percentage, there was no influence of the dietary NDF or ADF or of the RMY of the control group. In contrast, there was an interesting influence of the milk fat content of the control group (Figure 3). This figure shows that there is an increase in milk fat content in response to yeast supplementation when its value is low, suggesting the presence of subclinical acidosis.

**Discussion**

Several authors have reviewed the influences of yeast supplements on rumen digestion and animal performance (Ali Haimoud-Lekhal et al., 1999; Jouany, 1999; Lescoat et al., 2000; Garza-Cazares et al., 2001; Chaucheyras-Durand and Fonty, 2002; Robinson, 2002). However, except for the first cited paper, they were largely qualitative or narrative. Otherwise, several authors have noted that there was large variability among publications in the results of yeast influences on ruminant nutrition. That means that by selecting references, it is quite possible to conclude either a positive or a negative effect of adding yeast for any given parameter! Therefore, in order to obtain more relevant and reliable conclusions it was decided to perform a 2-step approach. The first step was to try to be as exhaustive as possible of the published results. The second step was to build databases in each area where a sufficient number of experiments were carried out and published. This is the origin of the four databases mentioned in this paper.

In the current work we had a clear confirmation of the large variability of data among papers. It must be considered that the present approach is only the first step of a more detailed and systematic analysis of all the specific factors which could explain, at least partly, the residual variations of the statistical models. Among these factors there are the strain and the actual level of yeast, comparison between yeast and fungi which were excluded in this work, methodological differences (batch vs continuous culture, adding yeast to the animal vs the fermentor, method of rumen sampling, etc.), dietary specifications, type of animal and level of dry matter intake, type of experimental design, etc. The major technical difficulties that we met in this work were fairly classical, the tables of data were largely

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**Figure 3.** Influence of control milk fat content (MFC) on MFC change with the addition of *S. cerevisae* in dairy cows.
incomplete for our purposes and the experimental methodologies and conditions varied largely from one publication to another. Moreover some other specific difficulties were met, the major one being the fact that several of the experiments were available only in form of abstracts and that most of those were very incomplete. This situation can induce bias in the meta-analysis, because non-significant results are generally not presented in an abstract. For a further step, it would be very important to have more complete information on all these studies. Another problem was that another group of the publications dealing with digestion was very incomplete with, for example, poor information on the specifications of the diet, or substrate used or on the animal characteristics. All these aspects mean that there was obviously a large range in the ‘quality value’ of the various published experiments dealing with yeast response. This suggests that it would be useful to re-run the statistical treatment by weighting the experiments using a ‘quality index’. Another way of weighting the data would be to take into account the residual variations of each experiment. For all these reasons, whatever the conclusions that could be now drawn, it is very important to consider that the all the results presented here cannot be considered as definitive. Lastly, it is probable that non-positive or non-significant results had a lower probability of being published than positive or significant trials, which induced a bias in the conclusions.

The method of meta-analysis that we have used is now considered the most suitable (St Pierre, 2001). It is of interest to study the nutritional impact of rumen defaunation (Eugene et al., 2004) and to quantify the biases of rumen mechanistic models (Offner and Sauvant, 2004). This method of statistical modeling has the advantage of splitting variation among and within experiments. The latter is therefore controlled and cannot interfere with the former. However, as such, the meta-design and the model cannot allow us to easily extract and analyze the interactions between the experimental influences and the impact of adding yeast. Obviously this last issue is a big one in the current context. Another big issue in meta-analyses is the way to treat the aberrant treatments and (or) experiments. For the present it was decided to include all the data in the process of interpretation. However it was decided to indicate the proportion of treatments that could be considered aberrant. Moreover, it was also decided to indicate the probability level of the test because in the context of the meta-analysis the threshold value of significance is of importance.

The results were not systematically consistent from one database to another. Some comments have already been noted in the text on this aspect. In the in vitro data it was fairly clear that pH was increased, suggesting a preventative role of yeast toward acidosis. The results were not so clear in vivo, however they were globally consistent. Moreover the interference of either the percentage of concentrate, or the dietary NDF, rapidly degradable carbohydrates, cannot be easily tested due to the scarcity of experiments targeted for that purpose and due to the lack of dietary specification. On this aspect the in vivo database with dairy cows provided an interesting observation, which suggested that yeast supplementation is more efficient in restoring milk fat content when it is low, which is suggests subclinical acidosis.

Further detailed interpretation is also needed regarding VFA and lactic acid production and profiles. On this aspect the results of the in vitro simple culture could not be confirmed with mixed cultures or in vivo. As such, this paper cannot provide useful information about the influence of yeast on hydrogen status and redox aspects. On these points a more detailed interpretation will be necessary.

Microbial concentrations and activities were enhanced by yeast in co-culture and in mixed cultures. These data tended to be confirmed by others, such as the fibre degradability and digestibility data. However, there is a clear lack of in vivo data to confirm these observations on microbial activities. Experiments with duodenally-canulated animals could provide more accurate and extensive knowledge of the impact of yeast on quantitative metabolism in the rumen.

A mechanistic modeling approach was only considered at the level of the co-culture. The first results were encouraging; and it will be useful to develop a mechanistic model of the rumen to take into account the mode of action of yeast on some basic metabolic process such as lactate and VFA metabolism, microbial growth and activities. In this model it will be necessary to include the major principles of thermodynamics (Heijnen and Van Dijken, 1992). This aspect, which has been little explored, could help in understanding any influence of yeast on oxygen/hydrogen balance. As published today, the rumen mechanistic models present some strong limitations (Offner and Sauvant, 2004) and cannot be used to investigate these aspects. Therefore
such a project would allow significant progress in methods of modeling the nutritional impact of yeast and other dietary factors in ruminants.

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The top ten most frequently-asked questions about mycotoxins, cattle and dairy food products

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Introduction

Mycotoxins are toxic secondary metabolites produced by fungi (molds). Secondary metabolites are chemicals produced by the fungus that are not essential for growth. Mycotoxins are chemically diverse, represent a variety of chemical families, and range in molecular weight from c. 200 to 500. A practical definition of a mycotoxin is a fungal metabolite that causes an undesirable effect in exposed animals. The undesirable effect or disease caused by a mycotoxin is a mycotoxicosis (Nelson et al., 1993). Exposure is generally through consumption of contaminated feedstuffs, although dermal contact or inhalation of certain mycotoxins can also cause undesirable responses. Mycotoxins exhibit broad and variable biological effects in animals. Mycotoxins can cause damage to organ systems, reduce production and reproduction and increase disease by reducing immunity. Some mycotoxins are carcinogens. Some target the liver, the kidney, the digestive tract or the reproductive system. Symptoms are wide ranging including decreased feed consumption, poor feed utilization, weight loss, reduced performance, estrogenic effects, vomiting, diarrhea, nervous disorders, tissue necrosis, hemorrhage, tumors, abortions and death.

Do molds cause animal problems or do molds simply produce mycotoxins for that purpose?

There is no confirmed reason for the existence of mycotoxins. Most theories suggest that mycotoxins exist to protect or enhance the existence of the fungus. Recent speculation is that mycotoxins increase the ability of the mycotoxin-producing fungus to cause a plant disease, thus helping to create an environment conducive for growth of the fungus (CAST, 2003). In experiments, Fusarium graminearum and F. verticillioides were genetically altered so that they would not produce trichothecenes or fumonisins, respectively. Results were mixed, demonstrating that trichothecenes play an important role in wheat head blight and corn ear rot caused by F. graminearum (Desjardins and Hohn, 1997; Harris et al., 1999), but that fumonisins are not required for corn ear rot caused by F. verticillioides (Desjardins et al., 2002).

It is also possible that immune suppression in animals by certain mycotoxins is in fact a mechanism to allow infectivity by the fungus. Some fungi are infectious pathogenic agents that cause a mycosis (fungal infection) that has a detrimental effect on the host animal. Aspergillus fumigatus is thought to be a fairly common mold in both hay (Shadmi et al., 1974) and silage (Cole et al., 1977). Aspergillus fumigatus has been proposed as the pathogenic agent associated with mycotic hemorrhagic bowel syndrome in dairy cattle, which has also been attributed to Clostridial infections and other factors (Puntenney et al., 2003). Such mycoses occur in immunosuppressed animals. Dairy cows are immune suppressed in early lactation. Aspergillus fumigatus also produces a mycotoxin, gliotoxin, which is an immune suppressant. It is possible that immune suppression by gliotoxin is a mechanism that allows infectivity by the fungus. Gliotoxin was found in peritoneal lavages from mice inoculated and infected with A. fumigatus (Eichner et al., 1988). Gliotoxin has also been found in the udder of cows naturally infected with A. fumigatus, while other known mycotoxins produced by this fungus were absent.
The top ten most frequently-asked questions about mycotoxins, cattle and dairy food products

(Bauer et al., 1989). Interactions with trichothecene mycotoxins may also be a factor in occurrence of a mycosis because reductions in cellular immunity can reduce resistance to a mycosis. Niyo et al. (1988a, b), showed that rabbits exposed to T-2 toxin had a decrease in phagocytosis of *Aspergillus fumigatus* conidia by alveolar macrophages and an increase in severity of experimental aspergillosis. Richard (1991) has suggested that medical mycologists should consider this aspect of infections caused by any toxigenic fungus, especially those that produce immuno-suppressive compounds. Fungal pathogens include *Aspergillus fumigatus*, *Candida albicans*, *Candida vaginitis* and certain species of *Fusarium*.

Fungi are deterioration organisms. Therefore, feedstuffs on which they grow are deteriorated and have an altered nutritional value including decreases in fat, protein and carbohydrates, which can affect performance and health (DiConstantzno et al., 1995). Cook and Wu (1991) itemized some of the nutritional changes in feeds occurring with mold growth including a decrease in lysine and thiamin and an increase in fiber. Some of the interactions of mycotoxins with nutrients have been reviewed (Schaeffer and Hamilton, 1991).

How many mycotoxins exist – how frequently are they found?

Hundreds of mycotoxins have been identified, but other than the major mycotoxins, most have not been extensively researched and even fewer have good methods of analysis available. The major classes of mycotoxins are aflatoxins, zearalenone, trichotheccenes, fumonisins, ochratoxin A and the ergot alkaloids. These mycotoxins are the more likely causes of mycotoxicoses in dairy cattle and other domestic animals because they occur more frequently and have the potency to cause toxicities. However, there are many reports of mycotoxicoses that have occurred as a result of those mycotoxins that are categorized as minor in importance (CAST, 2003; Lacey, 1991).

Riley (1998) put forth an argument that only a small proportion of mycotoxins have yet been identified. One factor that supports this idea is the high rate of discovery of new mycotoxins. To further support this idea, Riley (1998) cited the following facts, which we have taken the liberty to condense. Riley (1998) noted that Hawksworth (1991) estimated that there may be as many as 1.5 million fungal species in the world, but only about 69,000 are currently identified. Turner (1978) and Turner and Aldridge (1983), cataloged 3200 secondary metabolites produced by 1600 fungal species, or two secondary metabolites per fungal species. Cole and Cox (1981) classified 10% of the fungal secondary metabolites as mycotoxins. If there are indeed 1.5 million fungal species and if each produces two secondary metabolites and if 10% of secondary metabolites are mycotoxins, there are potentially 300,000 mycotoxins. More conservatively, if there are 100,000 fungal species producing 200,000 secondary metabolites, there may be 20,000 mycotoxins in nature. Potentially, many more mycotoxins exist than have been identified.

The potentially large number of unidentified mycotoxins and the fact that commercial laboratory analyses are not available for many of the identified mycotoxins suggests that a mycotoxicosis can occur without any possibility of identifying the mycotoxin, or all of the mycotoxins, that may be interacting to produce the mycotoxicosis.

The frequency of the occurrence of mycotoxins and the proportion of feeds that are contaminated indicate that animal exposure is high. The FAO has suggested that 25% of the world’s crops are affected (CAST, 1989). Certainly raw agricultural products are more likely to be contaminated than the human food supply. Mycotoxins are present worldwide with some geographical differences mainly resulting from climatic differences. There are differences in mycotoxins by type of feedstuff. Occurrence and concentrations are variable by year, which is expected because of the annual variation in weather conditions and plant stresses known to affect mycotoxin formation (Coulumbe, 1993). Summaries of surveys showing the incidence and concentrations of mycotoxins in various feedstuffs have been published (CAST, 2003; Wood, 1992; Wood and Trucksess, 1998). Feed samples submitted by North Carolina farmers over a 13 year period (Table 1) indicate that mycotoxins in feeds occur commonly at unsuitable concentrations (Whitlow et al., 1998). It can be concluded that mycotoxins occur frequently in a variety of feedstuffs and are routinely fed to animals.

What conditions support mold growth and mycotoxin formation?

Perhaps the major mycotoxin-producing fungal genera, in terms of research in the United States, are
Aspergillus, Fusarium, and Penicillium. Many species of these fungi produce mycotoxins in a variety of feedstuffs. Claviceps spp. particularly in small grains and Epichloe and Neotyphodium in fescue grass all produce ergot alkaloids. These fungi and their mycotoxins are also a concern, but have a more specific host-fungal relationship than do Aspergillus, Fusarium, and Penicillium fungi. Molds are fungi that grow in multicellular colonies, as compared with yeasts that are single cellular fungi. Molds can grow and mycotoxins can be produced pre-harvest or during storage, transport, processing, or feeding. Mold growth and mycotoxin production are related to weather extremes (causing plant stress or excess hydration of stored feedstuffs), to inadequate storage practices, to low feedstuff quality, and to faulty feeding conditions. In general, environmental conditions - heat, water, and insect damage - cause plant stress and predispose plants in the field to mycotoxin contamination. Because feedstuffs can be contaminated pre-harvest, control of additional mold growth and mycotoxin formation is dependent on storage management. After harvest, temperature, moisture content, and insect activity are the major factors influencing mycotoxin contamination of feed grains and foods (Coulombe, 1993).

Molds grow over a temperature range of 10-40°C (50-104°F), a pH range of 4 to 8, and above 0.7 a_w (equilibrium relative humidity expressed as a decimal instead of a percentage). Mold can grow on feeds containing more than 12-13% moisture. In wet feeds such as silage, higher moisture levels help exclude air and molds grow only if oxygen is available. The conditions most suitable for mold growth may not be the optimum conditions for mycotoxin formation in the laboratory. For example, the Fusarium molds associated with alimentary toxic aleukia have been reported to grow prolifically at 25-30°C without producing much mycotoxin, but at near-freezing temperatures large quantities of mycotoxins were produced with minimal mold growth (Joffe, 1986). Field applications of fungicides may reduce mold growth, in turn reducing the production of mycotoxins. However, the stress or shock of the fungicide to the mold organism may cause increased mycotoxin production (Boyacioglu et al., 1992; Gareis and Ceynowa, 1994).

Aspergillus species normally grow at lower water activities and at higher temperatures than do the Fusarium species. Therefore, Aspergillus flavus and aflatoxin in corn are favored by the heat and drought stress associated with warmer climates. Penicillium species grow at relatively low water activities and low temperatures and are widespread in occurrence. Penicillium molds are more common in storage than in preharvest, but can grow in the field under very wet conditions. Because both Aspergillus and Penicillium can grow at low water activities, they are considered storage fungi (Christensen et al., 1977).

Growth of A. flavus can occur at 86-87% equilibrium relative humidity (RH) (Davis and Diener, 1983). Field infection of corn with A. flavus (Wicklow, 1983) is expected when temperatures, including nighttime temperatures, are high and there is drought stress. Growth conditions in the southern US result in routine aflatoxin contamination of crops, but aflatoxin can be found in crops grown in other regions in years when weather conditions are conducive. For example, 8% of samples of midwestern US corn grain from the 1988 drought season contained aflatoxin (Russell et al., 1991). Corn is susceptible to A. flavus infection via the silks (Marsh and Payne, 1984) and stress conditions at the time of anthesis (pollination) lead to preharvest aflatoxin contamination in corn. A. flavus spores as inoculum are plentiful at this time. In North Carolina, insect activity appears less important in the events leading to aflatoxin contamination of corn than it appears to be in Georgia (Payne, 1983). Aflatoxin is a greater problem in cottonseed grown in the southwestern US than in the southeastern US (Ashworth et al., 1969). The complex effects of relative humidity, temperature, precipitation, and their daily variations may interact to produce conditions conducive to A. flavus infection and aflatoxin production in the Southwest (Ashworth et al., 1969). Early harvest and a decrease in late-season irrigation

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>Aflatoxin</th>
<th>Deoxynivalenol</th>
<th>Fumonisin</th>
<th>T-2 Toxin</th>
<th>Zearalenone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low, % of samples below (concentration)</td>
<td>6.4 (&lt;20 ppb)</td>
<td>18.2 (&lt;500 ppb)</td>
<td>32.6 (&lt;5000 ppb)</td>
<td>1.5 (&lt;100 ppb)</td>
<td>7.1 (&lt;300 ppb)</td>
</tr>
<tr>
<td>High, % of samples above (concentration)</td>
<td>4.0 (&gt;19 ppb)</td>
<td>28.2 (&gt;499 ppb)</td>
<td>9.4 (&gt;4999 ppb)</td>
<td>6.6 (&gt;99 ppb)</td>
<td>8.3 (&gt;299 ppb)</td>
</tr>
</tbody>
</table>
may reduce contamination (Russell et al., 1976). Experimentally, the use of spores of nontoxigenic A. flavus isolates in southwestern cotton fields has resulted in greatly reduced aflatoxin levels in cottonseed (Cotty et al., 1994). Improperly stored cottonseeds are susceptible to mycotoxin contamination if mold activity is allowed.

The *Fusarium* species are generally considered to be field fungi and were thought to proliferate before harvest (Christensen et al., 1977). However, *Fusarium* species may also grow and produce mycotoxins under certain storage conditions. In corn, *Fusarium* molds are associated with ear rot and stalk rot, and in small grains, they are associated with diseases such as head blight or commonly referred to as scab (Tuite et al., 1974). *Fusarium* is associated with excessive moisture at flowering. In corn, *Fusarium* diseases are more commonly associated with a cool wet growing season, with insect damage, warm conditions at silking, and wet conditions late in the growing season (Trenholm et al., 1988).

Of the *Fusarium* species *F. graminearum* is a major producer of deoxynivalenol (DON) and zearalenone (ZEN), but other species of *Fusarium* also produce DON and ZEN, as well as other mycotoxins (Christensen et al., 1988; Marasas et al., 1984). Conditions exacerbating ZEN accumulation in corn include weather that holds moisture content at 22-25%, or delayed harvest (Abbas et al., 1988). Zearalenone has been reported to occur in corn, other grains, and silage in many areas of the world. Weathered soybeans have also been reported to be contaminated with ZEN (Hagler et al., 1989). ZEN is also found in wheat, barley, oats, sorghum, sesame seed, hay, and silages. DON occurs in cereal grains worldwide and can increase in stored grain with kernel moisture contents of 22-25%. Minimum tillage and no tillage production are believed to increase the amount of disease in small grains and corn/wheat rotations because of increased inoculum survival on crop residue (Trenholm et al., 1988). T-2 toxin is produced primarily by *F. sporotrichioides* and *F. poae*, but is also produced by other species of *Fusarium* (Marasas et al., 1984). T-2 (and DAS) is often found in barley, wheat, millet, safflower seed, and in mixed feeds.

**How is a mycotoxicosis diagnosed?**

Hamilton (1978) presented an interpretation of the application of Koch’s postulates to mycotoxins. The postulates as modified are:

1. Find the mycotoxin in suspect substrate from the toxicosis outbreak.
2. Find in the substrate a fungus that produces the toxin.
3. Induce the toxicosis in experimental animals by ingesting or contacting the toxin.

Once the mycotoxicosis is established as a disease entity, it is no longer necessary to repeat the process. Recognition of the mycotoxicosis symptoms and mycotoxin presence in feed provide an adequate basis for diagnosis. If symptoms unique to that mycotoxin are observed, then it is not necessary to determine that the mycotoxin is in the feed.

Mycotoxins result in a progression and diversity of symptoms that can be confusing and can make diagnosis difficult (Hesseltine, 1986; Schiefer, 1990). Symptoms from field cases can be different from those observed under controlled experimental conditions because in field cases there may be multiple mycotoxins, variable dosages at irregular intervals, uncontrolled environments, and various interacting stress factors. Diagnosis is complicated by a lack of research, by a lack of feed analyses, by numerous possible mycotoxins, by nonspecific symptoms, and by immunosuppression resulting in opportunistic diseases that produce confounding symptoms. Therefore, a definitive diagnosis of a mycotoxicosis is difficult from general symptoms, specific tissue damage, or even feed analyses. However, experience with mycotoxin-affected herds greatly increases the probability of recognizing a mycotoxicosis. A process of elimination of other factors, coupled with feed analyses and unique symptoms can help identify a mycotoxicosis. Another practice helpful in diagnosis is an observation of positive responses or alleviation of symptoms after the use of products known to be effective in reducing mycotoxin exposure to animals. Examples of such products are mold inhibitors and mycotoxin sequestering agents. Regardless of the difficulty of diagnosis, mycotoxins should be considered as a possible cause of production and health problems when pertinent symptoms exist and problems are not directly attributable to other typical causes (Schiefer, 1990).

**How do mycotoxins affect dairy cows?**

Mycotoxins can increase incidence of disease and
reduce production efficiency. Some of the gross effects of mycotoxins can include: 1) intake reduction or feed refusal, 2) reduction in nutrient absorption and metabolism, 3) digestive disorders including hemorrhage and necrosis, 4) tissue and organ damage, 5) gangrene of the extremities, 6) endocrine effects, 7) reproductive disorders, embryonic death, abortions, 8) nervous disorders, tremors, uncoordination, 9) suppression of the immune system, and 10) death. Symptoms will be dependent on the mycotoxins present. In the field, animals experiencing a mycotoxicosis may exhibit a few or many symptoms. They may simply be unthrifty, with a rough or dull hair coat, have an undernourished appearance, impaired reproduction, and/or a mixed infectious disease profile. Some of the symptoms observed with a mycotoxicosis may be secondary, resulting from an opportunistic disease that is present because of immune suppression caused by the mycotoxin exposure.

Toxicity occurs at the cellular level. Aflatoxin causes DNA changes, cell deregulation, cellular changes and death. Deoxynivalenol inhibits protein synthesis resulting in disruption of cytokine regulation, altered cell proliferation and cell death. T-2 toxin inhibits protein synthesis with subsequent cell death. Fumonisin alters enzyme activity, which disrupts lipid metabolism resulting in cell deregulation and cell death. Zearalenone binds with cytosolic estrogen receptors causing an estrogenic response and altering hormonal control (Riley and Norred, 1996).

AFLATOXINS

Aflatoxins are a family of extremely toxic, mutagenic, and carcinogenic compounds produced by Aspergillus flavus and A. parasiticus (Deiner et al., 1987; Kurtzman et al., 1987). Toxigenic A. flavus isolates produce aflatoxins B1, and B2 and toxigenic A. parasiticus isolates produce aflatoxins B1, B2, G1, and G2 (Cotty et al., 1994).

Symptoms of acute aflatoxicosis in mammals include inappetence, lethargy, ataxia, rough hair coat, and pale, enlarged fatty livers. Symptoms of chronic aflatoxin exposure include reduced feed efficiency and milk production, icterus, and decreased appetite (Nibbelink, 1986). Reduced growth rate may be the only clue for chronic aflatoxicosis and other mycotoxicoses (Raisbeck et al., 1991; Pier, 1992). The mechanism by which aflatoxins reduce growth rate is probably related to disturbances in protein, carbohydrate and lipid metabolism (Cheeke and Shull, 1985).

Depending on interactions with other factors, aflatoxin concentrations as low as 100 ppb may be toxic to dairy and beef cattle, however the toxic level is generally considered to be between 300 to 700 ppb. Garrett et al. (1968) showed an effect on weight gain and intake with diets containing 700 ppb aflatoxin, but if increases in liver weights are used as the criteria for toxicity, then 100 ppb would be considered toxic to beef cattle. Guthrie (1979) showed a decline in reproductive efficiency when lactating dairy cattle in a field situation were consuming 120 ppb aflatoxin. When cows were changed to an aflatoxin-free diet, milk production increased over 25%. Patterson and Anderson (1982) and Masri et al. (1969) also suggest that 100 ppb may reduce milk production.

Aflatoxin produced from culture was shown to be more toxic to dairy cattle than pure aflatoxin added to diets (Applebaum et al., 1982). This is thought to result from other mycotoxins present in the natural culture. Under certain conditions, A. flavus also produces sclerotia, or resting bodies, which contain indole alkaloids such as aflatrem (Wicklow, 1983). Cyclopiazonic acid (CPA), a toxic indole tetratic acid, is also produced by A. flavus (CAST, 1989). The role of these and other toxins produced by A. flavus in aflatoxocoses is not known. Aflatoxin lowers resistance to diseases and interferes with vaccine-induced immunity in livestock (Diekman and Green, 1992).

The Food and Drug Administration (FDA) has established nonbinding action levels as informal guidelines for enforcement of aflatoxin control in feedstuffs (Table 2, Wood and Trucksess, 1998). Blending contaminated ingredients with uncontaminated ingredients with the purpose of reducing aflatoxin concentrations is not allowed.

| Table 2. US Food and Drug Administration action levels for total aflatoxins in food and feed. |
|---------------------------------|------------------|
| Food or feedstuff               | Concentration (ppb) |
| All products, except milk, designated for humans | 20 |
| Corn for immature animals and dairy cattle | 20 |
| Corn and peanut products for breeding beef cattle, swine, and mature poultry | 100 |
| Corn and peanut products for finishing swine (>100 lb) | 200 |
| Corn and peanut products for finishing beef cattle | 300 |
| Cottonseed meal (as a feed ingredient) | 300 |
| All other feedstuffs | 20 |
| Milk | 0.5 * |

ZEARALENONE

Zearalenone and zearalenol are estrogenic metabolites of several species of *Fusarium*. Chemically, zearalenone (ZEN) is a resorcylic acid lactone which does not have actual toxicity. Zearalenone is the cause of hyperestrogenism, the estrogenic syndrome, in swine. *F. graminearum* is the major ZEN-producing fungus of the *Fusarium* species that cause corn ear and stalk rots, but other species of *Fusarium* produce ZEN, as well as other mycotoxins (Christensen *et al.*, 1988).

Zearalenone is rapidly converted to α– and β-zearalenol in rumen cultures (Kiessling *et al.*, 1984). α–Zearalenol is c. four-fold more estrogenic in rats than ZEN, while β-zearalenol is about equal in strength to ZEN (Hagler *et al.*, 1979). However, ZEN has been considered of less importance to ruminants. Ruminal conversion of ZEN was found to be about 30% in 48 hrs (Kallela and Vasenius, 1982). A controlled study with nonlactating cows fed up to 500 mg of ZEN (dietary concentrations of about 40 ppm ZEN) showed no obvious effects except that corpora lutea were smaller in treated cows (Weaver *et al.*, 1986a). In a similar study with heifers receiving 250 mg of ZEN by gelatin capsule (dietary concentrations of 25-30 ppm ZEN), conception rate was depressed about 25%; otherwise, no obvious effects were noted (Weaver *et al.*, 1986a). Several case reports have related ZEN to an estrogenic response in ruminants and sometimes included abortions as a symptom (Kallela and Ettala, 1984; Khamis *et al.*, 1986; Mirocha *et al.*, 1968; Mirocha *et al.*, 1974; Roine *et al.*, 1971). Other cattle responses may include vaginitis, vaginal secretions, poor reproductive performance and mammary gland enlargement of virgin heifers. In a field study (Coppock *et al.*, 1990), diets with about 750 ppb ZEN and 500 ppb DON resulted in poor consumption, depressed milk production, diarrhea, and total reproductive failure. New Zealand workers (Towers *et al.*, 1995a,b; Sprosen and Towers, 1995; Smith *et al.*, 1995) have successfully estimated intake of ZEN and its metabolites (ZEN+M) by measuring urinary ZEN and its metabolites which include zearalanone, α- and β-zearalenol and α- and β-zearalanol. ZEN+M intake predicted from urinary ZEN+M was associated with reproductive disorders in sheep and dairy cattle. In sheep, ZEN+M was related to lower conception, reduced ovulation, increased twinning rates and a 10 to 20 % decline in fertility of ewes. With dairy cattle, herds with low fertility had higher levels of blood and urinary levels of ZEN+M. Individual cows within herds, examined by palpation and determined to be cycling, had lower blood ZEN+M levels than did cows that were not cycling. The reproductive problems in dairy cattle were associated with ZEN+M concentrations of about 400 ppb in the pasture samples.

TRichoTHECENES

Trichothecenes are a family of 200-300 related compounds that apparently exert their toxicity through protein synthesis inhibition at the ribosomal level. Several species of *Fusarium* and related genera produce trichothecenes. T-2 toxin, diacetoxyscirpenol (DAS), and DON are commonly found in agricultural commodities (Desjardins *et al.*, 1993). However, except for DON, it appears that most contamination with T-2 toxin and DAS occurs post-harvest. The toxic effects of trichothecenes include gastrointestinal effects such as vomiting, diarrhea, and bowel inflammation. Anemia, leukopenia, skin irritation, feed refusal, and abortion are also common. The trichothecenes, as a group, are immuno-suppressive (Sharma, 1993).

DEOXYNIVALENOL

The impact of DON on dairy cattle is not established, but clinical data show an association between DON contamination of diets and poor performance in dairy herds, but without establishing a cause and effect (Whitlow *et al.*, 1994). DON may therefore be a marker for low-quality mycotoxin-contaminated feeds in these herds. Other case reports help substantiate an association of DON with poor performing dairy herds (Gotlieb, 1997 and Seglar, 1997). DON has been associated with reduced feed intake in nonlactating dairy cattle (Trenholm *et al.*, 1985). There was a trend (P<0.16) for a 13% loss in 4% fat corrected milk in a study utilizing 18 midlactation dairy cows (average 19.5 kg milk), consuming diets shown to contain no common mycotoxins other than DON which was at levels of approximately 0, 2.7 and 6.5 ppm in treatment diets (Charmley *et al.*, 1993). Noller *et al.* (1979) used 54 lactating dairy cows in a 3 x 3 Latin square experiment with 21-day feeding periods. *Gibberella zeae* (*F. graminearum*) infected corn was used to provide estimated concentrations of 0, 1650 and 3300 ppb
DON and 0, 65 and 130 ppb of ZEN in three experimental diets. While neither intake nor milk production (22.9 kg/d) were affected, cows that received contaminated grain gained significantly less weight. Conversely, Ingalls (1996) fed lactating cows diets containing 0, 3.6, 10.9 or 14.6 ppm of DON for 21 days, without an apparent effect on feed intake or milk production (30 kg/d). DiCostanzo et al. (1995), in a review of several individual studies, concluded that beef cattle and sheep can tolerate up to 21 ppm of DON without obvious deleterious effects.

The FDA had provided an advisory for DON concentrations in wheat and wheat-derived products (Table 3) (Wood and Trucksess, 1998).

T-2 TOXIN

T-2 toxin is produced primarily by *F. sporotrichioides* and *F. poae*, but is also produced by other species of *Fusarium* (Marasas et al., 1984). Data with cattle are limited, but the toxicity of T-2 toxin in laboratory animals is well-documented (Wannemacher et al., 1991). T-2 toxin is a very potent mycotoxin associated with gastroenteritis, intestinal hemorrhages (Petrie et al., 1977; Mirocha et al., 1976) and death (Hsu et al., 1972; Kosuri et al., 1970). T-2 toxin fed to cattle at 0.64 ppm for 20 days resulted in death and bloody feces, enteritis, and abomasal and ruminal ulcers (Pier et al., 1980). Kegl and Vanyi (1991) observed bloody diarrhea, low feed consumption, decreased milk production and absence of estrus cycles in cows exposed to T-2. Weaver et al. (1980) showed that T-2 was associated with feed refusal and gastrointestinal lesions in a cow, but did not show a hemorrhagic syndrome. Serum immunoglobulins and certain complement proteins were lowered in calves receiving T-2 toxin (Mann et al., 1983). Gentry et al. (1984) demonstrated a reduction in white blood cell and neutrophil counts in calves. A calf intubated with T-2 developed severe depression, hindquarter ataxia, knuckling of the rear feet, listlessness and anorexia (Weaver et al., 1980).

**FUMONISINS**

This family of mycotoxins is produced by the species of *Fusarium* in the Liseola section. *F. verticilloides* (formerly *F. moniliforme*), a species that is almost ubiquitous in corn, and *F. proliferatum* are the main species producing high yields of fumonisins. Fumonisins B₁, B₂, and B₃ (FB₁, FB₂, and FB₃) are produced in fungal cultures or found in naturally contaminated corn samples (Cawood et al., 1991). Feed infected with *F. verticilloides* has long been associated with outbreaks of blind staggers (equine leukoencephalomalacia, ELEM) in equines (Wilson et al., 1985). Fumonisin B₁ was first isolated in South Africa where *F. moniliforme* has long been associated with animal problems (Gelderblom et al., 1988). Fumonisins has been shown to cause leukoencephalomalacia in horses (Marasas et al., 1988), pulmonary edema in swine (Harrison et al., 1990) and hepatotoxicity in rats (Gelderblom et al., 1991). Fumonisins are structurally similar to sphingosine, a component of sphingolipids. Sphingolipids are in high concentrations in myelin and in certain nerve tissues. Fumonisin toxicity is thought to result from disruption of sphingolipid biosynthesis (Riley et al., 1996). A USDA, APHIS survey of 1995 corn from Missouri, Iowa and Illinois found that 6.9% contained more than 5 ppm fumonisin B₁ (Anon., 1995). Murphy et al. (1993) reported fumonisin concentrations in corn for the Iowa, Wisconsin, and Illinois crops. Incidence of contamination was greater than 60% and concentrations ranged from 0 to 37.9 ppm. Corn screenings contained c. 10 times the fumonisin content of the original corn.

<table>
<thead>
<tr>
<th>Product</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All finished wheat products, e.g. flour, bran and germ, for human consumption</td>
<td>1</td>
</tr>
<tr>
<td>Grains and grain by-products destined for ruminating beef cattle and cattle in feedlots older than 4 months and for chickens (these ingredients should not exceed 50% of the diet)</td>
<td>10</td>
</tr>
<tr>
<td>Grains and grain by-products destined for swine (these ingredients should not exceed 20% of the diet)</td>
<td>5</td>
</tr>
<tr>
<td>Grains and grain by-products for all other animals (these ingredients should not exceed 40% of the diet)</td>
<td>5</td>
</tr>
</tbody>
</table>

1Wood and Trucksess, 1998
While FB₁ is thought to be much less potent in ruminants than monogastrics, work by Kriek et al. (1981) suggested that fumonisin was toxic to sheep. Osweiler et al. (1993) fed young steers 15, 31 or 148 ppm fumonisin in a short term study (31 days). There were no significant effects on feed consumption or gain; however, there was a trend toward lower intake and weight gains for those fed 148 ppm. With the highest feeding level, there were mild liver lesions in calves, and the group had elevated liver enzymes indicative of liver damage. Lymphocyte blastogenesis was significantly impaired at the end of the feeding period in the group having the highest dose.

Dairy cattle (Holsteins and Jerseys) fed diets containing 100 ppm fumonisin for approximately 7 days prior to freshening and for 70 days thereafter demonstrated lower milk production (6 kg/cow/day), explained primarily by reduced feed consumption. Increases in concentrations of serum enzymes suggested mild liver disease (Diaz et al., 2000). Dairy cattle may be more sensitive to fumonisin than are beef cattle, perhaps because of greater production stress.

Fumonisin has been shown to be carcinogenic in rats and mice (NTP, 1999), and has been associated with esophageal cancer in humans in China (Chu and Li, 1994) and South Africa (Rheeder et al., 1992). Therefore, fumonisin contamination has implications for human health, at least from a regulatory perspective. The FDA released guidance for fumonisin levels in human foods and animal feeds in late 2001 (Table 4).

### OCHRATOXIN A

This mycotoxin is produced by species of Penicillium and Aspergillus, and is a causative agent of kidney disease in pigs that has been referred to as mycotoxin porcine nephropathy, producing symptoms including diarrhea, increased water consumption, diuresis and dehydration (Krogh, 1979). OTA is rapidly degraded in the rumen and thus thought to be of little consequence unless consumed by young pre-ruminant calves (Sreemannarayana et al., 1988).

### CITRININ

Citrinin can co-occur with OTA, is produced by both Penicillium and Aspergillus, and like OTA targets the kidney (Kitchen et al., 1977). Symptoms of pruritis, pyrexia and hemorrhagic syndrome in a dairy herd were attributed to citrinin (Griffiths and Done, 1991).

### PATULIN

Patulin is produced by Penicillium, Aspergillus, and Byssochlamys and may be found in silage (Dutton et al., 1984; Hacking and Rosser, 1981). Patulin has been incriminated as a possible toxin in Europe and New Zealand (Lacey, 1991).

### PR TOXIN

Produced by Penicillium roquefortii, PR toxin has

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**Table 4. US FDA guidance for industry on fumonisin levels in human foods and animal feeds.**

<table>
<thead>
<tr>
<th>Human foods</th>
<th>Total fumonisins (FB₁+FB₂+FB₃) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Product</strong></td>
<td></td>
</tr>
<tr>
<td>Degermed dry milled corn products</td>
<td>2</td>
</tr>
<tr>
<td>Whole or partially degermed dry milled corn products</td>
<td>4</td>
</tr>
<tr>
<td>Dry milled corn bran</td>
<td>4</td>
</tr>
<tr>
<td>Cleaned corn intended for masa production</td>
<td>4</td>
</tr>
<tr>
<td>Cleaned corn intended for popcorn</td>
<td>3</td>
</tr>
<tr>
<td><strong>Animal feeds</strong></td>
<td></td>
</tr>
<tr>
<td>Corn and corn by-products intended for:</td>
<td></td>
</tr>
<tr>
<td>Equids and rabbits (no more than 20% of diet)</td>
<td>5</td>
</tr>
<tr>
<td>Swine and catfish (no more than 50% of diet)</td>
<td>20</td>
</tr>
<tr>
<td>Breeding ruminants, breeding poultry and breeding mink and including lactating dairy cattle and hens laying eggs for human consumption (no more than 50% of diet)</td>
<td>30</td>
</tr>
<tr>
<td>Ruminants ≥3 months old being raised for slaughter and mink being raised for pelt production (no more than 50% of diet)</td>
<td>60</td>
</tr>
<tr>
<td>Poultry being raised for slaughter (no more than 50% of diet)</td>
<td>100</td>
</tr>
<tr>
<td>All other species or classes of livestock and pet animals (no more than 50% of diet)</td>
<td>10</td>
</tr>
</tbody>
</table>

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2 Limits on ingredients are on a dry weight basis.
been found in silage (Hacking and Rosser, 1981) and was the suspected vector in a case study with symptoms of abortion and retained placenta (Still et al., 1972). Surveys of grass and corn silage in Europe have found *P. roquefortii* in up to 40% of samples (Auerbach, 2003).

DICOUMAROL

Dicoumarol is produced from natural plant compounds when *Penicillium* or *Aspergillus* molds grow on sweet clover or sweet vernal grass. Dicoumarol interferes with the function of vitamin K, resulting in a hemorrhagic syndrome. Moldy sweet clover poisoning is discussed by Radostits et al. (1980).

ERGOT ALKALOIDS

One of the earliest recognized mycotoxicoses is ergotism caused by a group of ergot alkaloids. They are produced by several species of *Claviceps*, which infect the plant and produce toxins in fungal bodies called sclerotia or ergots. Ergotism primarily causes a nervous or gangrenous condition in animals. Symptoms are directly related to dietary concentrations and include reduced weight gains, reduced milk production, and agalactia (Robbins et al., 1986). Sclerotia concentrations above 0.3% are related to reproductive disorders. Fescue infected with *Neotyphodium* or *Epichloë* may contain toxic alkaloids associated with ‘fescue toxicity’ (CAST, 2003). Fescue is a major pasture grass in the US, growing widely throughout the lower midwest and upper south. Over half of the fescue is endophyte-infected, making this a serious problem for cattle and horse producers. Endophyte-free varieties are available, but they are not as hardy as infected varieties. Fescue infected with a nonpathogenic endophyte may be more field hardy and less toxic.

Are dairy products contaminated when dairy cattle consume mycotoxins?

Moy (1998) reviewed the international efforts to evaluate and reduce the human risks of mycotoxins. He stated that “human health problems caused by the consumption of most mycotoxins are complex and poorly understood”, but they may be responsible for a range of diseases. The majority of human health risk from mycotoxins is from consumption of contaminated grains and nuts. While many mycotoxins are common contaminants of feedstuffs and several mycotoxins have been shown to occur in the milk of dairy cattle, concentrations are extremely low because only a small fraction of the amount consumed by a cow is transferred to milk in the parent form or as a derivative. Aflatoxin is the only mycotoxin that has received regulatory action in the US as a possible contaminant in milk. This is because aflatoxin transfer from feed to milk is greater than for other mycotoxins. Also, aflatoxin is carcinogenic, highly toxic to humans, and because milk is a primary component of the diet of infants. The US FDA indicated that aflatoxin is the only mycotoxin that currently warrants regulation in milk (Wood and Trucksess, 1998).

AFLATOXIN

Milk aflatoxin residues are the result of transformation of the parent compound in the liver and its subsequent secretion into milk. Aflatoxin B₁ results in milk residues of aflatoxin M₁, while aflatoxin B₂ results in milk residues of aflatoxin M₂. Small amounts of other derivatives such as aflatoxin M₃, Q₁, and aflatoxicol can also be found in milk; however aflatoxin M₁ is the primary residue (Wood, 1991). Van Egmond (1989) concluded that aflatoxin carryover from feed to milk is approximately 1-2%. Frobish et al. (1986) found greater aflatoxin transfer to milk when the toxin was supplied by contaminated cottonseed meal than when it was supplied by contaminated corn. Percentage transfer of aflatoxin to milk was not affected by concentration in the feed or by milk production level of the cow. They concluded that concentration of aflatoxin M₁ in milk was approximately equal to 1.51% of the concentration of aflatoxin B₁ in the diet. Therefore a concentration of 33 ppb in the total diet would result in a 0.5 ppb concentration in milk (3.9 ppb in the milk dry matter, assuming 12.8% milk solids). Figure 1 shows the extent to which four toxin adsorbents added to the diet of dairy cows reduced aflatoxin M₁ in milk (Diaz et al., 1999).

Regulatory pressures and a widespread awareness have helped minimize aflatoxin problems. Surveys of aflatoxin B₁ concentrations in feedstuffs conducted during the 1980s resulted in lower levels than for surveys conducted in the 1970s (Van Egmond, 1989).
The United States General Accounting Office (GAO, 1991) concluded that industry, federal and state programs are effective in detecting and controlling aflatoxin and that it is doubtful that additional programs or limits would reduce the risk of aflatoxin in the food supply. The GAO specifically examined the state-administered program in the state of Georgia as a part of its report. In 1989, 13% of corn samples tested by the Georgia Department of Agriculture exceeded 20 ppb. On farms, 3.9% of tested milk exceeded limits while at the retail level only 0.4% of milk was in violation. Current surveillance programs in the US aimed at reducing food residues make it very unlikely that aflatoxin will be fed at high enough levels and for sufficient duration to have significant production or health effects on dairy herds in those regions that have an active program.

Dairy cattle feeds should contain less than 20 ppb aflatoxin to prevent milk residues above 0.5 ppb. Concentrations of aflatoxin should be conservatively low because of uncertainties in sampling and analysis, nonuniform distribution of aflatoxin, and potential for more than one source of aflatoxin in the diet.

DEOXYNIVALENOL

DON is changed to DOM-1 in the rumen with estimates of 24 hr degradation of about 50% (King et al., 1984). Deoxynivalenol and metabolites are rapidly excreted, primarily through urine (Côté et al., 1986; Prelusky et al., 1984; Prelusky et al., 1987). Prelusky et al. (1984) administered DON in an oral dose of 920 mg and found less than 4 ppb of free and conjugated DON in the milk. DON was excreted in milk primarily as DOM-1, but excretion rate is extremely low at 0.0001% of the dose. Côté et al. (1986) found no DON, but up to 30 ppb of DOM-1 in milk of cows fed DON at about 300 mg/day (66 ppm) for five days.

ZEARALENONE

Shreeve et al. (1979) fed dairy cows about 1 ppm zearalenone for 11 weeks without detecting a milk residue. Prelusky et al. (1990) administered up to 6 g of zearalenone per cow daily and found a total milk residue of up to 16 ppb, which represented about 0.01% of the dose. Hagler et al. (1980) administered 5 g zearalenone in ground feed to a lactating dairy cow that was milked twice daily with samples collected until 120 hr after dosing. Only trace levels of zearalenone were found in the milk obtained at 96, 108 and 120 hr after dosing and trace levels of zearalenol were also found in the milk at 108 and 120 hr after dosing. Mirocha et al. (1981) found that zearalenone and its metabolites reached levels above 1 ppm in milk representing about 0.7% of the zearalenone dosage, which was 25 ppm for eight days.

T-2 TOXIN

Residues of T-2 and its derivatives have been found in milk, but have a low transfer rate from feed to
milk. After 72 hrs, an orally administered dose of T-2 at 0.42 mg/kg of body weight (approximately 36 ppm) was almost completely excreted in the feces and urine (Yoshizawa et al., 1981; Yoshizawa et al., 1982). Milk residues, which reached a maximum of about 35 ppb, suggest that about 0.2% of T-2 and its metabolites are secreted in milk. In the lactating cow administered radioactive labeled T-2 toxin, three metabolites (3'-hydroxy-T-2 toxin, 3'-hydroxy-HT-2 toxin and 3'-hydroxy-7-hydroxy-HT-2 toxin) accounted for 30-40% of the radioactivity in urine, 60-70% of radioactivity in milk and 50-60% of the radioactivity in blood plasma. Other metabolites included HT-2 toxin, neosolaniol and 4-deacetylneosolaniol. Other investigators (Robinson et al., 1979) have measured T-2 up to a peak of 160 ppb in milk on the fifth day after starting oral intubation with daily doses of 182 mg of T-2 toxin for 15 consecutive days (equivalent to about 9 ppm in the diet, assuming a daily consumption of 20 kg).

FUMONISIN

Fumonisin B₁ carryover from feed to milk is thought to be negligible (Richard et al., 1996; Scott et al., 1994). Prelusky et al. (1996) reported studies where dairy cattle were administered fumonisin B₁ either orally or intravenously. The oral dosages were approximately equal to dietary concentrations of 60 to 300 ppm. The intravenous dosages were stated to be similar to dietary concentrations of 125 to 500 ppm. No fumonisin B₁ or its metabolites were detected in milk (detection limit of 0.5 ppb for fumonisin B₁). Maragos and Richard (1994) analyzed 155 milk samples collected in Wisconsin during a period when feeds were reported to be severely affected by mold. Additionally, 10 samples were collected in Illinois. Feed samples associated with these milk samples were not collected and thus fumonisin B₁ concentrations in feed were unknown. Only one of the 165 milk samples tested positive for fumonisin B₁, which was determined to be 1.29 ppb. This suggests that fumonisin can occur in milk, but is likely to be at very low levels.

OCHRATOXIN

Goats were administered a single dose of radiolabeled ochratoxin A at 0.5 mg/kg (Nip and Chu, 1979). Cumulative excretion of radioactivity over seven days indicated that 53% was excreted in the feces, 38% in the urine and 6% in milk. Of the radioactivity in milk, only a small amount was in the form of ochratoxin A (OTA), representing 0.026% of the dosage administered. In a study with lactating cows where ochratoxin A was fed at 317 to 1,125 ppb for 11 weeks, neither ochratoxin A nor its metabolite ochratoxin α were detected in milk (Shreeve et al., 1979).

SUMMARY

Several other mycotoxins, or their derivatives, may be found in extremely small amounts in milk. Other than aflatoxin, they are not considered likely human health hazards in milk. It is thought that significant residues of these other mycotoxins occur in milk only when very high, nonclinical levels are administered to cows. Additionally the derivatives are generally less toxic than the parent compound. Aflatoxin is the only mycotoxin that has received regulatory action in the US as a possible contaminant in milk. Regulatory efforts have successfully reduced the risk of aflatoxin in the food supply in the US (GAO, 1991). Efforts to prevent aflatoxin formation, to divert contaminated ingredients away from dairy feeds usage, and to use feed additives that reduce aflatoxin absorption by the animal, have contributed to fewer milk contamination problems.

What are the safe levels of mycotoxins for dairy cattle?

Some of the same factors that make diagnosis difficult also contribute to the difficulty of establishing levels of safety. These include lack of research, sensitivity differences among animal species, imprecision in sampling and analysis, the large number of potential mycotoxins, and interactions with other mycotoxins and stress factors (Hamilton, 1984; Schaeffer and Hamilton, 1991). Mycotoxin effects are also moderated by factors such as sex, age, duration of exposure, and stresses of the environment and production. The known dietary factors that interact with mycotoxins include nutrients such as fat, protein, fiber, vitamins and minerals (Brucato et al., 1986; Coffey et al., 1989; Smith et al., 1971).

Naturally contaminated feeds are more toxic than feeds with the same level of a pure mycotoxin supplemented into the diet. Jones et al. (1982) demonstrated that productivity losses in commercial
broiler operations can occur when aflatoxin concentrations are below those shown by controlled research to be of concern in laboratory situations. Aflatoxin produced from culture was more toxic to dairy cattle than pure aflatoxin added to diets (Applebaum et al., 1982). In swine, Foster et al. (1986) demonstrated that a diet containing pure added DON was less toxic than diets with similar concentrations of DON, which were supplied from naturally contaminated feeds. Smith and MacDonald (1991) have suggested that fusicaric acid, produced by many species of *Fusarium*, occurs along with DON to produce more severe symptoms. Lillehoj and Ceigler (1975) give an example where penicillic acid and citrinin were innocuous in laboratory animals when administered alone but were 100% lethal when given in combination. These studies strongly suggest the presence of other unidentified mycotoxins in naturally contaminated feeds. It is well documented that several mycotoxins may be found in the same feed (Hagler et al., 1984). Abbas et al. (1989) demonstrated that *Fusarium* species isolated from Minnesota corn produced multiple mycotoxins. Because animals are fed a blend of feedstuffs and because molds produce an array of mycotoxins, many mycotoxin interactions are possible.

Interactions with other stress factors make recommendations difficult. Animals under environmental or production stress may show more pronounced symptoms. It is clearly shown that there is a temperature interaction with fescue toxicity such that more pronounced symptoms are expressed during heat stress (Bacon, 1995). Fumonisin at 100 ppm has been shown to reduce milk production in dairy cattle (Diaz et al., 2000), and in a separate study to not significantly affect average daily gain in beef cattle fed 148 ppm (Osweiler et al., 1993). While this contrast may reflect a difference in the duration of feeding, number of animals studied, etc., it may also suggest differences due to greater stress in early lactation dairy cattle as compared with young growing beef cattle.

Because of partial degradation in the rumen, mycotoxins are less toxic to cattle than to most other animals. However mycotoxins are not completely degraded and some of the degradation products remain toxic (Kiessling et al., 1984). Extent of ruminal degradation appears to be variable. It is speculated that feeding situations resulting in a faster rate of ruminal feed passage or a low population of protozoa in the rumen may reduce mycotoxin degradation in the rumen. Ruminal degradation of mycotoxins appears to be more dependent on protozoal than bacterial activity (Kiessling et al., 1984).

### Which feed mycotoxin source is the greatest problem - grains or silages?

Almost any type of feed can be contaminated with mycotoxins. Table 5 compares the incidence and concentrations of mycotoxins in corn grain and corn silage over a nine year period. Perhaps the worst case scenario for a dairy producer may be to have the on-farm stored feeds contaminated. Therefore, the season's supply of feed is contaminated and not just the current load that is used over a short time period. This is because toxicity is a function not only of amount but also duration of feeding. When the on-farm stored feed is contaminated, the dairy producer faces a difficult decision. The contaminated feed could be fed as normal, diluted with purchased feed or not used. Increased costs may be incurred from additional feed purchases or reduced income may result from a loss in animal performance. The contaminated feed may be either grain or forage; however, dairy producers are much more likely to store forage than grain. Therefore, it is important to discuss mycotoxin contamination of forages. Many different mycotoxins have been found to occur in forages either in the field, or in storage as hay or silage (Lacey, 1991). Some mycotoxicoses in cattle resulting from contaminated forages have been reviewed (Lacey, 1991; Gotlieb, 1997; Seglar, 1997; Whitlow, 1993; Whitlow and Hagler, 1997). It is unclear how much of the mycotoxin contamination of forages occurs prior to harvest. Fresh feed can be contaminated with mycotoxins at harvest; however, mold can grow in harvested forages. The limiting factor for mold growth in hay is low moisture content. When hay is stored too wet, mold is likely to grow, produce heat and cause heat damage. The limiting factors for mold growth in silage are a low pH and high moisture, which limit air infiltration. If silage is stored too dry or insufficiently packed and covered, infiltration of air allows for microbial activity which depletes silage acids, allowing pH to rise and molds to grow. Some of the *Penicillium* molds grow at a low pH (Auerbach, 2003). Silage on the feeding face must be removed at a rate that prevents air infiltration into the silage mass resulting in conditions that support mold growth. Practical recommendations are to feed 6 to 12 inches daily from the feeding face to prevent mold.
It appears that *Aspergillus flavus* does not grow well in hay or silage, however, aflatoxin concentrations up to 5 ppm have been reported (Kalac and Woolford, 1982). We have detected low levels of aflatoxin (<100 ppb) in corn silage and alfalfa. Table 5 shows that the frequency of aflatoxin in corn silage is not different from the frequency of aflatoxin in corn grain, but the concentrations are lower. The frequency and concentrations of some Fusarium-produced mycotoxins are also compared in Table 5. There is a trend toward a higher frequency of ZEN in corn silage than in corn grain.

*Aspergillus fumigatus* is thought to be a fairly common mold in both hay (Shadmi et al., 1974) and silage (Cole et al., 1977) and may be a vector in the development of mycoses in dairy cattle. Silage was found to contain fumigaclavine A and C and several fumitremorgens (Cole et al., 1977). Animal symptoms included generalized deterioration typical of protein deficiency, malnutrition, diarrhea, irritability, abnormal behavior and occasionally death. The hay was fed to goats and rats and resulted in retarded growth and histopathological changes in the livers and kidneys.

Surveys of grass and corn silage in Europe have found an occurrence of *P. roquefortii* in as many as 40% of samples (Auerbach, 2003). PR toxin, produced by *Penicillium roquefortii*, has been found in silage (Hacking and Rosser, 1981) and was the suspected vector in a case study with symptoms of abortion and retained placenta (Still et al., 1972). Vesely et al. (1981) reported that in cows fed corn silage containing PR-toxin there was loss of appetite, gut inflammation, rumen stasis and abortion. Moldy alfalfa hay containing *Aspergillus ochraceus* was implicated as producing OTA associated with abortions in cattle (Still et al., 1971). OTA in moldy forage has also been implicated in cattle deaths (Vough and Glick, 1993).

The most important pasture-induced toxicosis in the US is tall fescue toxicosis caused by endophytic alkaloids (Bacon, 1995). Other forage toxicoses of fungal origin include ergotism, perennial ryegrass staggers, slobbers syndrome (a hemorrhagic disease that is associated with dicoumarol produced in fungal infected sweet clover and sweet vernal grass), and syndromes of unthriftiness and impaired reproduction associated with *Fusarium* (Cheeke, 1995).

### How can mycotoxin effects be reduced?

Pre-harvest control has involved agronomic practices that minimize mycotoxin accumulation in the field. These include proper irrigation, pesticide application, resistant or adapted hybrids, proper tillage and fertilization. Unfortunately, breeding for mycotoxin-resistant hybrids has been only partially successful. Munkvold et al. (1999) have shown that compared with nontransgenic corn, *Bacillus thurengiensis* (Bt)-transgenic corn had less corn borer damage, less *F. verticillioides* infection, and lower fumonisin contamination. Fungicides have shown little efficacy in controlling pre-harvest aflatoxin contamination in corn (Duncan et al., 1994).

Post-harvest approaches for management of mycotoxin contamination include mycotoxin analysis of feedstuffs and diversion of contaminated lots; ammoniation of corn and cottonseed to destroy aflatoxin; dilution; and storage technology (Trail et al., 1995). Mycotoxin-contaminated grains can be used for ethanol production, and in some cases...
mycotoxin-contaminated grains can be diluted with clean feeds (Desjardins et al., 1993). The FDA does not allow dilution of aflatoxin-contaminated feeds, which is considered adulteration. The best strategy for post-harvest control of mycotoxins is proper storage and handling of feed grains.

Sampling and testing feeds is a part of a control program. The accurate determination of mycotoxin concentrations in grain and feeds depends on a number of factors. First, a statistically valid sample must be drawn from the lot (Whittaker et al., 1991). Because mycotoxins are not evenly distributed in grains and other feedstuffs, most of the error in a single analysis is due to sampling – as much as 90% of the error is associated with the taking of the initial sample. Proper collection and handling of representative feed samples is essential. Once collected, samples should be handled properly to prevent further mold growth. Wet samples may be frozen or dried before shipment and transit time should be minimized. The sample must then be finely ground and subsampled for analysis; this step is the second largest source of error in an analysis. Finally, the subsample is extracted, extract purified using one of several techniques, and then the toxin is measured. Toxin determination may be by thin-layer chromatography plates (TLC), high-performance liquid chromatography (HPLC), gas-liquid chromatography (GLC), enzyme-linked immunosorbent assays (ELISA), spectrophotometrically, or by other techniques. Blacklighting for bright-greenish-yellow fluorescence is often used as a screening technique for aflatoxin, but it is very inaccurate; newer and better methods should be used. As far as we are aware, blacklighting is completely inappropriate for other mycotoxins.

Mold spore counts may not be very useful and are only a gross indication of the potential for toxicity, but mold identification can be useful to suggest which mycotoxins may be present. Scott (1990) states that screening methods are needed for the Fusarium-produced mycotoxins and that one approach is to test first for DON, DAS, T-2 toxin and nivalenol, because other Fusarium mycotoxins seldom occur without one of these four also present. Feeds could then be tested for other mycotoxins if necessary.

Generally, laboratories provide analysis for only a limited number of mycotoxins, perhaps including aflatoxin, ochratoxin, deoxynivalenol, zearalenone, fumonisins, and T-2 toxin. Minimum detection levels may be limiting because they are often directed at finding high levels that cause serious animal disease, rather than low levels which are associated with production losses, impaired immunity and significant economic losses. However, analytical techniques for mycotoxins are improving, costs are decreasing and several commercial laboratories are available which provide screens for an array of mycotoxins. The Federal Grain Inspection Service (USDA-GIPSA) provides on the internet a list of approved mycotoxin tests for grains and provides excellent background materials for the feed industry (internet address http://www.usda.gov/gipsa/pubs/mycobook.pdf). Laboratory methods can be found in Official Methods of Analysis of AOAC International (Horwitz, 2000).

The potential for effective treatments has improved. Certain feed additives can reduce mycotoxin exposure of animals and thus minimize their negative effects. Some additives may be beneficial in reducing mycotoxin formation because they are effective in reducing mold growth. Ammonia, propionic acid, microbial and enzymatic silage additives have all shown some effectiveness as mold inhibitors. Additives to enhance fermentation can be added at ensiling. Mold growth inhibitors may be helpful as a surface treatment when capping off the silo or daily after silage feed-out to reduce molding of the exposed silage feeding face. If unacceptably high levels of mycotoxins occur, dilution or removal of the contaminated feed is preferable; however, it is usually impossible to replace all of a major forage ingredient. While dilution is sometimes a viable practice to reduce mycotoxin exposure, reduced feeding of silage could result in such a slow feedout, that mycotoxin problems within the silage increase. Ammoniation of grains can destroy some mycotoxins, but there is no practical method to detoxify affected forages already in storage. A microbial detoxification method (Binder et al., 2000) has been identified where a species of rumen bacterium (BBSH 797) was isolated which has the ability to biotransform DON, rendering it non-toxic. Field studies have suggested that the microbial product used as a feed additive can protect growing pigs from the effects of DON. Galvano et al. (2001) has reviewed dietary strategies to counteract mycotoxins. Increasing nutrients such as protein, energy and antioxidant nutrients may be advisable (Brucato et al., 1986; Coffey et al., 1989; Smith et al., 1971).

Sequestering agents such as clays (bentonites) added to contaminated diets fed to rats, poultry, swine and cattle have helped reduce the effects of mycotoxins (Diaz et al., 1997; Galey et al., 1987; Harvey, 1988; Kubena et al., 1993; Lindemann and Blodgett, 1991; Scheideler, 1993; and Smith, 1980; 1984). In most
cases, clay has been added to the diet at about 1%. Activated carbon at 1% of the diet effectively reduced aflatoxin in milk (Galvano et al., 1996). Activated carbon fed at 0.1% of the diet did not reduce aflatoxin levels in milk (Diaz et al., 1999). A glucomannan (Mycosorb®) fed at 0.05% of diet dry matter or bentonites at 1% of diet dry matter were similarly effective in reducing aflatoxin concentrations in milk (Diaz et al., 1999) (Figure 1). The low inclusion rate of the glucomannan in comparison to clay-type absorbants may be an important difference. A recent review of mycotoxin binders provides more details, but only limited comparison data are available (Huwig et al., 2001).

What policy changes and research are needed?

The CAST (2003) publication listed a number of important needs for research and public policy. We have summarized a few of those. It is obvious that current information and understanding of mycotoxins is inadequate; and for animal agriculture there is a critical need for better methods of diagnosis, prevention and treatment.

1. Ensure a safe food supply.
2. Develop uniform worldwide standards and regulations for mycotoxin contamination.
3. Improve mycotoxin analyses to be more definitive, quicker, simpler and cheaper.
4. Develop better diagnostics to include biomarkers to detect animal exposure to mycotoxins.
5. Assess mycotoxins as virulence factors.
6. Investigate the immunosuppressing effects of mycotoxins.
7. Investigate the toxicological interactions of toxins with the host.
8. Investigate possible genetic differences in sensitivity to mycotoxins and the genetics of mycotoxin production by fungi.
9. Assess interactions among mycotoxins and with drugs, diet and nutrition.
10. Develop a better understanding of factors affecting mycotoxin formation in the field and in storage.
11. Improve understanding of the ecology and epidemiology of mycotoxin-producing fungi.
12. Develop sound agronomic management practices to reduce mycotoxin contamination.
13. Develop host-plant resistance to toxigenic fungi and to mycotoxin occurrence.
14. Develop better models to predict the potential for mycotoxin formation.
15. Develop better sampling protocols.

References

The top ten most frequently-asked questions about mycotoxins, cattle and dairy food products


248 The top ten most frequently-asked questions about mycotoxins, cattle and dairy food products


Food, nutrition and health
There has always been a gray area between art and science that is very difficult to define. Nowhere is this more apparent than in the development and preparation of food products. Master chefs, bakers, candy makers (e.g.) have for centuries been able to present cuisine that routinely defies the ability of a scientist to reproduce. The nutritional aspects of the scientist’s food may be congruent and the ingredients utilized in the same ways, but the overall satisfaction and presentation of the chef’s creation is consistently lacking. Food, at the point of consumption, has a complex, integrated impact on the sensory organs. Taste, aroma, texture, appearance, time of day, state of health, age and mood can all affect the consumer’s experience and impressions of a given food. Of these variables, the one on which food scientists focus most of their efforts - using both science and culinary arts – is taste. Who doesn’t enjoy an evening in a five-star restaurant enjoying the talents of a master chef and who would not also enjoy the ability to buy from the local supermarket prepared products closely simulating the cuisine of a master chef? The food industry constantly attempts to bridge this gap between gourmet restaurant cuisine and ready-to-prepare off-the-shelf grocery products. Ingredients are being made and sold to assist in this process.

Among the key ingredients used to simulate savory flavors are hydrolyzed protein products. Proteins, as they undergo hydrolysis, develop definite ‘meaty’ taste characteristics that mankind has utilized for centuries. Initially the proteins undergoing hydrolysis were the meats themselves – especially beef – as they aged between time of slaughter and time of preparation. One of the fascinating observations on a busy main street in Buenos Aires is the large sides of beef hanging in the restaurant windows slowly aging to develop the highly desired taste of beef grown in the Pampas and cooked in the Argentine style. Another hydrolyzed protein system widely used for many centuries is soy sauce, which is made using a koji-type fermentation where the protease and α-amino peptidase enzymes of the *Aspergillus oryzae* culture organism break down the soy protein. As food ingredients, however, the use of hydrolyzed proteins did not become popular until about 100 years ago.

**Yeast extracts: creation and market development**

In the early 1900s as the populations of major cities in Europe began to grow with the Industrial Revolution, the minimal (or non-existent) sewage/waste treatment systems in the cities became overloaded. Companies were no longer permitted to just dump waste streams from manufacturing into the sewer. Among the big offenders of this dumping were the yeast companies. Grown on a high sugar nutrient source such as molasses, the resulting *Saccharomyces* yeast was sold to the bread makers and brewers for uses we all know well. When a batch was ‘out of spec’ regarding gas or ethanol production, it was simply discarded. Now, unable to just dump rejected batches, a few companies turned to scientists who discovered that the protease enzyme in the yeast would lyse cell proteins when the yeast cell died, resulting in a product with a desirable meaty flavor. Autolyzed yeast products were thus born and the market for them continues to grow to this day.

From this beginning yeast extracts of many types have been developed. For decades the terms ‘yeast extract’ and ‘autolyzed yeast’ were utilized interchangeably, but now the differences between them are clearly defined to facilitate label statements.
Autozyzed yeast has undergone the autolysis (protein hydrolysis) process but the insoluble yeast cell walls are still present. In contrast, a yeast extract has undergone the hydrolysis and the cell walls have been removed leaving a completely soluble, savory flavored product. The focus of this paper is yeast extracts, but we will briefly also consider the value of the cells walls, independent from the protein, as adjuncts for food and nutrition.

**Yeast extract manufacture**

The process of making yeast extracts is fairly straightforward; and in order to be cost effective, the key factor to control is cost of the yeast. No wonder then that the traditional manufacturers of yeast extracts are in either the baking yeast business (primary yeast) or operate breweries (spent yeast). The yeast cells are killed and the intracellular protease/peptidase enzymes start the process of digestion of the intracellular macromolecules (mostly protein and DNA). The hydrolysis of the protein proceeds until approximately 50-55% of the \( \alpha \)-amino linkages have been hydrolyzed. At this point, the hydrolysis is stopped using heat to denature the enzyme. Then the cell walls are ruptured, historically by adding a large amount of sodium chloride, so that the free amino acids and peptides created by the hydrolysis flow out into the supernatant. The cell walls are then removed via filtration; the remaining solution is concentrated and then dried. The resulting powder is very hygroscopic, so manufacturers previously added even more sodium chloride at this point to act as a flow conditioner. Now, however, fluid bed driers allow a more granular product with significantly reduced hygroscopic problems. Yeast extracts that contained 30-40% salt in the 1970s through the 1990s frequently contain 15-18% salt today. The result of this process is an off-white to brownish powder with a distinct, nondescript, meaty flavor note. This has considerable value when attempting to build a savory flavor profile.

Why not allow the hydrolysis to proceed beyond 50-55% of the \( \alpha \)-amino bonds in the yeast protein? We know that converting the yeast protein to 100% free amino acids would have more flavor impact per gram, but we also know that all enzymatic reactions are reversible. Consequently, as the hydrolysis proceeds, the point where the rate of hydrolysis and the rate of synthesis of \( \alpha \)-amino bonds are effectively equal is reached. Some of the peptides at this equilibrium are very bitter (objectionably so!); but at the 50-55% hydrolysis extent (measured as a ratio of \( \alpha \)-amino nitrogen:total nitrogen = 0.50 to 0.55) the desirable nondescript meaty flavor predominates.

**TYPES OF YEAST UTILIZED AND NEW SOURCES EMERGING**

Historically, four distinct groups of yeast have been explored for their value in yeast extract manufacture (listed in order of importance):

- **Primary baker’s yeast (Saccharomyces)** – grown on molasses.
- **Spent brewer’s yeast (Saccharomyces)** – grown on wort.
- **Torula yeasts (Candida)** – historically grown on paper or petroleum manufacturing wastes.
- **Dairy yeasts (Kluyvermyces)** – grown on sweet whey.

Of these, the primary baker’s and spent brewer’s yeasts currently account for the lion’s share of yeast extract manufacture. Large volumes of autolyzed torula are manufactured, but historically not converted to extracts and no company has ever successfully produced and sold extracts from \( \text{Kluyvermyces} \).

The types of yeast used are beginning to change somewhat. The availability and quality of brewer’s yeast has changed in recent years as large numbers of breweries in the traditional beer-making cities have closed, megabreweries have been built by the surviving companies and numerous types of beers – light, dry, traditional lager – are now made by these breweries. When the brewers were concentrated in cities such as Milwaukee, St. Louis, Cincinnati, etc., the manufacturers of the brewer’s yeast extracts could operate a centrally-located factory and get a regular, sustained supply of spent yeast that was usually only a few hours old when it arrived at the facility. Now these brewer’s yeast facilities tend to have one source located nearby but are forced to transport other supplies over long distances in order to operate near capacity. Although these transported lots are frequently chilled, the enzymatic processes that start following the death of the cell proceed; and the lot-to-lot variability of the end products made from these yeasts is increasing. The end user market for the brewer’s yeast extract group remains strong, but if a more reliable alternative with equal costing ever appears their use may decline significantly.
The manufacturers of yeast extracts from primary baker’s yeasts remain, with one notable exception, companies that make baker’s yeast for leavening as a core business. The baker’s yeast business has suffered in many parts of the world during the past three decades from over-capacity and price pressures, so the ability to shift capacity to yeast extract manufacture has been somewhat of a godsend for these companies. Thanks to the revelation that chloropropanols are present in acid hydrolyzed vegetable proteins, the use of primary baker’s yeast extracts grew at double-digit rates from the mid 1980s for about 15 years. Many end users however are now revisiting the risk-reward debate about the use of acid hydrolyzed plant proteins (HVPs) and some will switch back for cost reasons in the next 2-3 years. Because of the sustained rapid growth for such a long period of time, most of the key producers added capacity to cope and facilitate growth. When HVPs begin to supplant yeast extracts, much of this newer capacity will fall idle. Initially this will allow companies to retire aging, inefficient older facilities, but excess capacity will still exist. This could create price and profit pressures on the core producers at a time when their core business – yeast for baking operations – is also under considerable pressure. Where this group of products will be and the impact they will have in the market 20 years from now is difficult to predict.

New sources of yeast are looming as competitors and these may bring both price and yield advantages versus the existing yeast sources. These are Candida yeasts grown on non-waste substrates such as wood chips and sawdust and more significantly spent Saccharomyces recovered from the production of fuel ethanol. Candida presents a very attractive profile for making yeast extracts. The amino acids in the Candida protein consist of a high percentage of those desired for flavor development – glutamic acid, cysteine, methionine, aspartic acid – and the RNA levels are considerably higher than for Saccharomyces. A state-of-the-art manufacturing facility was recently constructed in Europe and primary growth Candida is now being produced on the sawdust, not the waste, coming from a paper manufacturing plant. The resulting yeast extracts have, depending on the manufacturing method utilized, either an excellent flavor profile or a very high level of 5’-active nucleotides. Currently the pricing on these products is somewhat aggressive and companies have been reluctant to switch; however, should prices become more competitive with primary baker’s yeast extracts, major users can be expected to change.

Long-term, the spent Saccharomyces from fuel ethanol production plants offer the potential for ‘clean-tasting’ yeast extracts (there are little, if any, off-flavor nuances from this production stream). Since this is a spent (or dead) yeast, production of the high nucleotide enhancer yeasts will not be practical (unless someone develops a technique to denature the intracellular DNA), but the use of extracts for base flavor production and Maillard reactions could significantly cut into the current market for both primary baker’s and spent brewer’s yeasts. These yeast cells will be available at almost zero cost to an ethanol producer and joint ventures between ethanol producers and food ingredient sellers are a highly likely event in both Brazil and the United States (and wherever else large volumes of ethanol production capacity are established). These products will almost certainly have a huge cost advantage on the primary baker’s yeasts now dominating the market and it is reasonable to assume that they will garner considerable market share in the next 20-25 years.

Building a savory flavor

THE CULINARY MODEL

Savory flavors are built in steps and have three primary parts: a flavor base, a specific flavor note, and enhancement. Using the culinary arts as the model, a chef would accomplish this as follows:

Base flavor note

Savory flavor bases are made using the bones, skin, fat and sometimes the organ meats of the animal taste desired. These raw materials are braised, roasted, slow cooked, etc. to develop flavor notes, then simmered for an extended period to extract those flavor notes and finally concentrated via reduction (evaporation). The chef then uses these savory flavor bases as the first key building block when developing a flavor for a given cuisine. When working at home in our own kitchens, we generally utilize bouillon cubes as the source of our base savory flavor notes.

Specific flavor note

The key specific savory flavor notes we generally desire are produced from Maillard reactions and the complex series of Amadori rearrangements and
Stecker degradations that follow. This is the highly desired roasted/cooked flavor we associate with the meat products we eat. When you and I cook at home we often see the brown film that forms on the bottom of the skillet or roasting pan as a cleanup headache, but the chef sees this as savory ‘gold’ and proceeds to ‘de glace’ the pan using wine, water, vinegar or whatever liquid is needed for the end product and possibly adding additional flavor notes as well (mushrooms, various vegetables or herbs).

**Enhancement**

Last is the process of giving the flavor as much impact as possible. This is done with enhancers. The ultimate savory flavor enhancer has always been sodium chloride, but there is a hierarchy of enhancers that all contribute to the satisfaction of the end result:

Salt
\[\equiv\]
Free glutamate
\[\equiv\]
Active nucleotides
\(5\prime\text{-IMP and } 5\prime\text{-GMP}\)
\(5\prime\text{-inosinate monophosphate and } 5\prime\text{-guanylate monophosphate}\)

Salt is a wonderful enhancer, but sodium intake levels have become a health concern in recent years. Salt content can be reduced if some free glutamate is also utilized. But glutamate has also routinely come under pressure from a small group of very vocal anti-MSG advocates who proclaim allergies to glutamate. A few people actually are very allergic, but most of the anti-MSG contingent are self-diagnosed. Many of the people who claim to have this allergy suffer reactions whenever they see MSG or hydrolyzed proteins in label statements, but surprisingly can enjoy pasta covered with a tomato-based sauce and parmesan cheese with no reaction at all. Lastly, both salt and glutamate can be reduced if active nucleotides are present.

Yeast extracts can contribute to two of the levels of enhancement. First, yeast protein is very high in glutamic acid. As the hydrolysis proceeds the levels of free glutamate become significant and the yeast extract can contribute to overall savory flavor enhancement. Second, live yeast cells contain fairly large amounts of RNA, which can be first enzymatically altered with deaminase (Aspergillus melleus) allowing deamination of adenosine to yield inosine. Secondly, enzymatic hydrolysis with ribonuclease (Penicillium citrinum) under very exacting conditions yields the 5’-active nucleotides.

Because RNA is quickly destroyed following the death of the yeast cell, these reactions must be done on live cultures. It has been done using primary yeast Saccharomyces cerevisiae for about 25 years and just recently this technology has been extended to primary Candida strains as well (RNA content is higher than for Saccharomyces). Interestingly, the ribonuclease enzyme does not attack the stable DNA molecules at all (yields are <0.5%), but can attack denatured, single strands of DNA with yields of approximately 85%.

To date, none of the yeast extract manufacturers are utilizing denatured DNA as a substrate for the manufacture of 5’-nucleotides, but it does suggest the potential to utilize spent Saccharomyces (from brewing and alcohol production) as a source of enhancer yeast extracts as well.

An interesting sidelight about active nucleotides is the fact they have an enhancement half-life in the mouth when consumed. When working in the lab developing new savory products it is important to keep this in mind. Sequential tasting of products causes an enhancement not only of the product containing the nucleotides, but also of the next samples in the queue. You can confirm this effect by making a control and an experimental sample to be tasted. Taste the control, taste the experimental and then taste the control again. Even rinsing the mouth with water and eating a soda cracker will not eliminate this effect. If true randomization is needed in savory panels, do the tests over a number of days with the three tasting samples in random order. Salt and glutamate do not have this effect. Rinsing the mouth and eating a soda cracker effectively eliminates their effect on the next savory flavor consumed.

**THE YEAST EXTRACT MODEL**

The intent of the food processor in developing a desirable flavor profile in a finished product is to mimic as closely as possible the culinary savory flavor model considered earlier. Again, the flavor profile will be built with three parts, all of which can be accomplished with yeasts although more frequently other ingredients are also combined with yeast extracts to produce the intended end result:

\[
\text{Flavor base} + \\
\text{Maillard-reacted specific flavor(s)} + \\
\text{Enhancement}
\]
This brings us to the three technologies behind the yeast extracts being manufactured and available in the market today: hydrolysis, secondary chemical processes and Maillard reactions.

**Degree of hydrolysis**

The first variable is the degree of hydrolysis, which is commonly measured by the $\alpha$-amino-nitrogen:total nitrogen (AN:TN) ratio. Since flavor impact requires free amino acids, the objective for yeast extracts in flavor development is hydrolyze to the fullest extent possible without producing bitter flavor notes. Typically this occurs at an AN:TN ratio of 50-55.

When the objective is flavor enhancement however, the goal is to minimize the degree of hydrolysis (some hydrolysis is needed for complete solubility of the extract). After the desired flavor has been achieved, we do not want to alter that flavor when we enhance it; rather, we just wish to make the impact of that flavor more pronounced. The AN:TN ratios for these extracts are typically between 10 and 20. At this level of hydrolysis the extract will have very little flavor impact and will also tend to be quite light in color.

**Secondary chemical processes**

The other key variable is the secondary changes (other than protein hydrolysis) either prior to or after the hydrolysis steps. We have already considered one of these, the production of active 5´-nucleotides, which is typically done only on enhancer (low degree of hydrolysis) extracts prior to the actual hydrolysis. These products now rival the volume of the traditional yeast extracts in terms of usage in the market, but when they were first developed there was little enthusiasm for their use. Initially, the developers attempted to make a product that had a high degree of hydrolysis and contained the active nucleotides. This was generally unsuccessful since yields of the nucleotides declined during the protein hydrolysis processing time, so they reduced the degree of hydrolysis, got levels of active nucleotides that would be effective as enhancers and told their sales force to go sell the product. Nothing happened! The yeast extract sales force, business people with no technical backgrounds, went to their core customers and showed the product simply as a new yeast extract. The customers tried it, reported there was insufficient flavor impact and discontinued its use. It remained an R&D curiosity for over six years and probably would still be such today if chloropropanols had not been discovered in acid hydrolyzed plant proteins (HVPs). During a 30-year span from approximately 1955 to 1985, HVPs were developed from wheat gluten, corn gliaden and numerous other plant protein sources. The hydrolysis reaction was conducted under elevated temperature and pressure using 0.1N HCl with hydrolysis proceeding to almost 100%. The hydrolysate was then neutralized using 0.1N NaOH, filtered and dried. These HVPs, given the higher degree of hydrolysis, had more flavor impact and were more cost effective than yeast extracts, so the major soup, sauce and snack manufacturers switched away from yeast extracts. For cost reasons, the HVP manufacturers could not use ‘pure’ proteins as substrates, so small amounts of the lipid fraction of the plant source were also present. When researchers found chloropropanols (carcinogens) present in numerous HVP products at parts per million levels, many of the market leaders using HVPs made the commitment to minimize risk by switching to the enzymatically hydrolyzed yeast extracts. One of the effects of this was a marked decrease in flavor impact in the finished products. Consequently, they started looking for ‘label-friendly’ ways to enhance their products and the high nucleotide yeasts finally were ‘discovered’. A period of exponential growth followed.

**Maillard reactions**

There is also a post-hydrolysis processing step that creates the crucial specific flavor note products that are needed if we want to create the entire finished savory flavor profile from a yeast source – Maillard reactions. Flavor manufacturers have increasingly utilized these reactions to manufacture natural savory flavors since the late 1940s, but the practice is still not widely utilized by the yeast extract manufacturers. Yeast extracts are an excellent source of the amino acids that produce these savory flavor notes and a very wide range of specific flavors can be developed using an extract as the only nitrogen source and combining it with different reducing sugars. Some of the flavors possible include:

- General brown roasted flavor
- Roast beef
- Roast chicken
- Roast pork
- Chocolate
- Nutty flavors
- Bread
Much of the primary baker’s yeast extract sold today goes to the major flavor manufacturers who use it as a preferred source of α-amino nitrogen for further reactions. Bitterness created during the hydrolysis process is not necessarily a problem in this application, but to date none of the extract manufacturers have pursued making an extract with maximized degree of hydrolysis aimed at the flavor reaction and fermentation markets.

Numerous factors affect the results from Maillard reactions:

Proportion and nature of the reactants. Generally, the sugar has less influence on the sensory properties of the final flavor than the amino acid. Pentoses are generally more reactive than hexoses. Lipids, if present, can also interact in the Maillard reaction to modify the volatile aroma compounds present (Farmer and Mottram, 1990).

Solubility. Aqueous solubility can play an important role. For most substances, except gases, solubility increases with increasing temperature. Reactions occurring in phases are significantly slower than those occurring in a single phase.

Solvent. Changing the solvent not only affects the rate of reaction, it can change the outcome of the reaction as well (Heinze, 2002). To date, this has been poorly understood and solvent use is a closely guarded trade secret.

Water activity. Highest yields for model systems have been reported at a water activity of 0.72 (Hartman et al., 1984). Generally the results will show boiled meat flavor notes at higher water activities and more roasted meat flavor notes at lower water activities.

pH. Affects the rate of reaction when the reactions take place in an aqueous environment (Heinze, 2002).

Temperature. Changing temperature not only affects the rate of reaction, it also tends to change the product distribution in the finished flavor as well (Heinze, 2002).

Time. Both length of the reaction and pre-reaction aging (this can be significantly modified using enzymes) affect the outcome.

Developing these flavors and learning to scale them up to industrial processes requires a great deal of trial and error. Flavor chemists generally learn this by working initially as a bench technician for a more senior colleague and then ultimately getting assignments as their experience develops. The end results are seen every day in the foods we buy, both in the grocery store and at fast food type restaurants, but the processes and substrates for making them are closely guarded corporate secrets. There are a couple of flavor courses available to learn about these processes, one of which includes lab development.

**FORMULATING THE FLAVOR SYSTEM - THE STARTING POINT**

A flavor is typically built in steps. First a base flavor is combined with a specific flavor(s) to approximate the flavor profile desired in the end product. Typically the base component will constitute 50-80% of the blend and the specific flavor(s) 20-50% of the blend. The flavors made can be tested using a taste panel and costs calculated to determine their acceptability and then altered as needed:

![Diagram showing base flavor, specific flavor, and target flavor](image)

- More flavor impact – increase the ratio of specific flavor to base flavor
- Lower cost – increase the ratio of base flavor to specific flavor(s)

In general, the specific flavor products made entirely from yeast will cost between two and ten times the cost of the base flavor components.

Once the desired flavor profile is achieved, then it can be added to the finished product and the overall impact evaluated. When a savory flavor is made entirely from yeast sources, it almost always requires some enhancement. Remembering that adequate levels of salt and free glutamate must be present (the yeast extracts in the flavor system will usually furnish enough free glutamate), the enhancer yeast extract can then be added to get the flavor’s impact in the final product to the desired intensity. Generally the starting ratio for this will be about 90% of the
compounded flavor and 10% of the high nucleotide enhancer yeast.

Theoretically, you now have a finished product, with an added flavor system made entirely from yeast that the market will adopt and buy like mad because it tastes so good!

Outside the box - what else might we be able to do with yeast?

To date, there are only two manufacturers of yeast extracts making extracts as their primary business. All the others are either growing yeast to sell as an active leavening agent or to use up a waste stream from their core business. The use of both the β-glucan and mannan fractions of the yeast have been under study for some time in human nutrition and their efficacy as probiotics is now pretty much accepted. Other authors at this symposium are addressing their nutritional benefits in non-human mammals. At present there is a race for use patents regarding these ingredients in human foods. We will, at some point, see the complex carbohydrate fractions and the hydrolyzed protein fraction becoming sufficiently valuable that some company(s) will discontinue yeast sales for baking and just make the derivative pieces noted above.

Also, genetic engineering of single cell organisms is fairly easy and offers the potential to make savory flavor development even better. Steps, either through strain selection or genetic code insertions that can create higher levels of the sulfur-containing amino acids, glutamic acid or higher levels of RNA will yield strains of yeasts even more valuable for flavor development. If we no longer have to concern ourselves with the CO₂ production or ethanol production of a strain, then we can look at changing the chemical composition of the yeast cell to be more savory flavor friendly.

Bon apetite

Nutritional objectives are difficult to force onto consumers. People eat what they like, not what is good for them. The cereal manufacturers, for example, have tried for two decades to build more nutrition into their cereals. Consumers even say they want this when responding to panel inquiries. But we continue to buy what our families like, not necessarily what is good for them. Making a culinary taste for a mass-produced food product is one of the ways to get people to eat better, more nutritious diets. We have become a population with serious weight problems. Social factors are partly to blame, but excess calories are as well. The food producers and the restaurant companies see this, too. There is a real effort right now to bring better quality with lower serving sizes to the market. Yeast extracts and the flavor systems assist this process of making the key savory portions of meals more attractive and more culinary. Bon appetite, hopefully we can eat for both health and enjoyment, not one or the other.

References

One university’s search for intelligence in a universe of foods for wellness

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Introduction

The students in our universities this year are likely to see the world population double in their lifetime and up to 20% of the earth’s species threatened (Population Council, 2004; McKee et al., 2004). Meanwhile, the emission of heat-trapping gases is predicted to cause global climate changes. To solve these problems, the experts agree that students need to think in terms of patterns and systems, while our universities are organized around disciplinary specialization. Universities educate students as if art, science, ethics and the long-term future of humanity were unrelated. Planning a future for food requires that universities institutionalize the capacity to think and act across discipline boundaries as if culture, science, agriculture, ethics, and the long-term were all related.

The problems of the next generation will have considerable impact on providing a safe and adequate supply of appealing, health-promoting foods. An idealistic mission for an academic center focusing on foods for wellness might be to build consensus for how an institution for food research will invest its limited resources for integrating food, health and agriculture in the short and long terms. Consensus would be based on sound science, but would also be subject to humanities, ethics, social sciences, and complete knowledge. Typically, consensus begins with prior research and builds on prior success.

A university-based visioning process begins with campus food and agricultural leadership and extends outward until it exerts an influence on public opinion. This influence is the sum of its faculty and their graduates, their activities in professional organizations and government, and their public and private collaborators. Their influence used to be primarily regional, but is now also national and international. Consumer-driven industries and government agencies follow public opinion when enacting policy. Examples of public influence on food policy are the Dietary Supplements Health and Education Act of 1994, the USDA organic certification regulation, and the reluctance of large food companies to market controversial foods. Consumers have demonstrated that they will accept policy based on tradition, cultural beliefs, food ethics, and incomplete science, while they turn to health professionals, universities and the media for food and health information.

To understand how education can serve food systems, the University of California, Davis, located in the nation’s top agricultural state, serves as a model for critical examination. It is important, however, to provide the context in which UC Davis serves its food and agricultural mission.

The state of California

California’s two leading commodities in cash receipts are milk and grapes. California produces about 20% of the milk receipts and 91% of the grape receipts in the nation. California is ranked first in the US in the production of fluid milk, butter, ice cream and nonfat dry milk and second in cheese (CA Dairy Research Foundation, 2003). The American Dietetic Association claims that nine in ten women believe calcium is important to their health (ADA, 2003), yet the same number of women over age 30 consume only about half the amount of calcium recommended per day (Institute of Medicine, 1997). In California, as in many developed countries, consumption of nutrients does not correlate to the availability of the agricultural sources of those nutrients, although...
improving yield has always been a primary goal of agricultural research.

Agriculture in California encompasses over 350 crops. California food processors are the primary US producers of dried and dehydrated fruits and vegetables, and also produce a wide assortment of convenient fresh and frozen fruits and vegetables. It is the only state producing many specialty foods such as almonds, artichokes, raisins, prunes, olives, dates, figs and pistachios. The state supplies 45% of the world’s supply of processed tomato products; 100% of the nation’s canned peaches and fruit cocktail; and 100% of the supply of black ripe olives (California League of Food Processors, 2004). These crops have been supported by 50 years of food science and agricultural research at UC Davis. In the 1920s, when fatalities from botulism in preserved olives threatened the olive industry, UC developed the canned olive preservation process that was later adapted to many varieties of canned fruits and vegetables. California had to become a leader in preservation technology, since it is the leading agricultural export state and the sixth largest exporter in the world.

Table 1. California’s top food commodities – 2003.

<table>
<thead>
<tr>
<th>Product</th>
<th>Value (million US$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk and cream</td>
<td>3,812</td>
</tr>
<tr>
<td>Grapes</td>
<td>2,579</td>
</tr>
<tr>
<td>Lettuce</td>
<td>1,278</td>
</tr>
<tr>
<td>Cattle and calves</td>
<td>1,229</td>
</tr>
<tr>
<td>Almonds</td>
<td>1,190</td>
</tr>
<tr>
<td>Strawberries</td>
<td>991</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>926</td>
</tr>
<tr>
<td>Oranges</td>
<td>559</td>
</tr>
<tr>
<td>Broccoli</td>
<td>488</td>
</tr>
<tr>
<td>Carrots</td>
<td>460</td>
</tr>
<tr>
<td>Chickens</td>
<td>452</td>
</tr>
<tr>
<td>Avocados</td>
<td>358</td>
</tr>
<tr>
<td>Pistachios</td>
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</tr>
<tr>
<td>Potatoes</td>
<td>307</td>
</tr>
<tr>
<td>Walnuts</td>
<td>305</td>
</tr>
<tr>
<td>Lemons</td>
<td>287</td>
</tr>
</tbody>
</table>


As urban growth squeezed farming out of populated areas, some of the large food processors were squeezed out of California by urban sprawl, environmental regulations, energy costs, labor costs, consolidation, or all of the above. The processors that remain are tied to the state by geographic proximity to agricultural production. Campbell Soup and La Victoria Foods use locally grown tomatoes and vegetables for their soups, ‘V-8’ juice and salsa. California-grown rice, corn and potatoes are ingredients in snack foods made by Quaker Oats and Frito-Lay. California milk goes into Kraft and Land-o-Lakes cheese and Haagen Dazs ice cream. Blue Diamond and Hershey’s are the nation’s largest users of California almonds. Dole Vegetables uses California lettuce and carrots. Most of these companies have expanded their California operations in the last ten years.

California food manufacturing benefits from proximity to consumers. California’s population in the 2001 census was 34,501,130 and climbing (US Census Bureau, 2004).

California’s agriculture is concentrated in the Central Valley, which is also home to 47 endangered animal species and 44 endangered plant species. Some of the species are found throughout the Central Valley – others are found in only one county (Umbach, 1997). Agriculture is favorably viewed by California conservation groups as a means to provide habitat, as illustrated by the partnership between rice growers and local duck clubs. San Francisco’s regional watershed reached an agreement with local cattle ranchers to continue cattle production in San Mateo County. The Nature Conservancy maintains cattle ranching on its properties. California’s Marin County, immediately north of San Francisco, established a farmland trust (AFT, 2004) that served as a model for the American Farmland Trust, which is hosting their annual conference in Lexington, KY in November, 2004. According to a May 2001 report by the Agricultural Issues Center of UC Davis, the state lost approximately 500,000 acres of farmland to urban development between 1988-1998.

The University of California

The ten campuses of the University of California lead the nation in developing new patents (439) and have for the previous ten years, according to a the US Patent and Trademark Office. More than 1,000 California R&D-intensive companies actively engage in research projects with UC scientists and students. UC trains two-thirds of California’s doctors. One of every three California R&D firms was founded by UC scientists. It is estimated that 85% of the nation’s biotechnology industry employs UC alumni with graduate degrees. UC awards about 3000 PhDs every year. UC also serves agricultural and community development through 650 scientists at its Agricultural Experiment Station research labs and UC Cooperative Extension county offices throughout the state.
UC DAVIS AND THE CALIFORNIA INSTITUTE OF FOOD AND AGRICULTURAL RESEARCH

UC Davis is the largest campus in the UC System at 5300 acres. It features a relative abundance of space for growth compared to campuses in Los Angeles, San Diego or San Francisco, however the City of Davis has resisted residential growth, keeping prices high and forcing students and staff to commute from surrounding communities. The campus has plans for a residential community on agricultural land, reflecting statewide population pressures on agricultural land use. Enrollment this academic year is 30,229 undergraduate and graduate students and will be capped next year due to budget constraints. Ethnicity is 42% white, 27% Asian/Pacific, 10% Chicano/Latino, 2% African American; and 55% female. Research funding in 2002-2003 was $426 million public and $72 million private. The campus functions much like a city with its own public transportation system, police and fire services, waste treatment, and energy generation.

My view on the campus is from the California Institute of Food and Agricultural Research (CIFAR), founded in 1991 as a self-supporting program in the College of Agricultural and Environmental Sciences (CAES). CIFAR’s vision is to ensure that UC Davis remains connected to solving food and agricultural problems. Our mission is to create and enhance channels for research collaboration, program sponsorship and technology exchange between UC Davis, government and private food and agricultural organizations. We deliver results through communication, scientific and community involvement and networks. We inform faculty and industry participants through trend forecasting, conferences, targeted demonstration and research projects, and forums. Our program serves as a dynamic catalyst for linking strategically applied disciplines: foods for health; advanced food processing and packaging; water and energy management; and biomass resource utilization. A current list of our sponsoring organizations from industry and government can be found on our web site (CIFAR, 2004).

FOOD AND HEALTH RESEARCH AT UC DAVIS

The search for intelligence in a ‘universe’ of foods for wellness tracks food from farm to fork, or more accurately from farm to sewer. In 1994, CIFAR recognized food for wellness as an emphasis on campus. CIFAR’s executive director, Sharon Shoemaker, featured nutrition research at semiannual conferences and provided programmatic overviews of food research activities for visitors. Our early conferences, like those of other campuses, tried to explain what these functional foods were, but we were never comfortable with the ambiguous nature of the term. As interest in this area has expanded, the task has become exponentially larger in terms of the numbers of programs adding intelligence every year. Every biological department and school on campus has its own established research collaborations in the food system. Even though multidisciplinary grants and large proposals are becoming strategic to funding, it is not uncommon for two UC Davis investigators to be funded by the same source, and not be aware of it until a chance meeting at a professional conference in another part of the world.

The College of Agricultural and Environmental Sciences (CAES) is a platform that builds a basis for the intelligence about food and its role in health through 21 departments, 9 centers, and 40 majors that teach topics such as: agricultural economics, agronomy, animal science, food and agricultural engineering, aquaculture, avian science, chemistry, physical chemistry, enology, environmental sciences, environmental toxicology, food crop development, food product design, food processing, food engineering, fermentation science, food safety and regulation, food chemistry, human and community development, nutrition, pomology, risk/benefit assessment, range science, vegetable crops, viticulture, and sensory and consumer sciences.

The UC Division of Biological Sciences has six sections and nine majors that collaborate with CAES on topics relating to food production such as microbiology, cellular biology, biochemistry and molecular biology, plant physiology, plant pathology, exercise biology, neurobiology, physiology, behavior and genetics. The Schools of Medicine and Veterinary Medicine are very active in nutrition research and public health research. The Graduate School of Business Management emphasizes the food, health and beverage businesses. UC Davis hosts the US Department of Agriculture (USDA) Western Human Nutrition Research Center, with 50 scientists, many of whom have adjunct appointments in nutrition and medicine at the University. The UC Cooperative Extension system provides outreach for all these activities statewide.

The breadth of the resources of social and science intelligence inherent in these organizations, and the
numbers of individuals that might participate in an integrated initiative to improve health appears at first glance to be unmanageable. In addition to CIFAR, campus resources available to further research related to food and health include: the Biotechnology Program; Sustainable Agriculture Research and Education Program; bioinformatics; computer programming; chemical and statistical analysis services; the primate center, technology transfer, and UC Connect, a campus link to venture capital and business development services.

The UC Davis Genetic Resource Collections serve as depositories of many sources of food and knowledge regarding their production: fruit and nut trees, tomato germplasm, yeast, seeds, insects, rootstocks, domestic avian species, oysters, fish, bacteria, viruses, plants and herbs (GRCP, 2004). The campus news service plays a vital role in disseminating food/health research to the public through their link to the national media, to UC broadcasts and web casts, and through their training of food researchers in media communications skills.

Table 2. Food crop breeding programs at UC Davis¹.

<table>
<thead>
<tr>
<th>Alfalfa</th>
<th>Cherry</th>
<th>Peach</th>
<th>Prune</th>
<th>Wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almond</td>
<td>Chestnut</td>
<td>Pear</td>
<td>Rice</td>
<td>Walnut</td>
</tr>
<tr>
<td>Apricot</td>
<td>Fig</td>
<td>Pistachio</td>
<td>Strawberry</td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>Grape</td>
<td>Plum</td>
<td>Vegetables</td>
<td></td>
</tr>
<tr>
<td>Bean</td>
<td>Nectarine</td>
<td>Potato</td>
<td></td>
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</table>

¹UC Davis Technology Transfer Center.

ACADEMIC CENTERS OF DEVELOPMENT OF FOODS FOR WELLNESS

The core elements for an integrated UC Davis vision for the identification and development of health-promoting foods are in place. Yet, UC Davis has moved more slowly, and perhaps more thoughtfully than other campuses to define how a comprehensive center might be developed, what it would offer and to whom, and who would direct and participate in its activities. The goal of most centers is to further research collaboration and find creative ways to strengthen the web of connections between disciplines. In order to exploit those connections to ‘further intelligence’, links to the interests of the public must be apparent. A secondary goal, and perhaps a more urgent one in the short term, is to use that web of connections to compete for funding. Two university centers that have obtained funding this way are the Center for Designing Food to Improve Nutrition, Iowa State University, Ames; the Center for Enhancing Foods to Protect Health at Purdue, and The Functional Foods for Health Program at the University of Illinois.

The UC Davis CAES initiative for foods for health is taking an inventory of its core competencies to define a short-term direction. Exploring differentiation and areas of core competence is as necessary to a university as to a business. Universities evaluate themselves in terms of intellectual resources, physical resources, accomplishments, alumni, and collaborations, compared with those of its competitors. Defining core areas of expertise is necessary for attracting federal and state funding, but also for gifts, corporate contracts, and endowments. The funding success that can be achieved through this exercise can be illustrated by the UC Davis Cancer Center.

The UC Davis Health System invested $70 million in the cancer program over the past decade, recruiting 35 new research scientists and building a new Cancer Center and state-of-the-art cancer research facility. Last year the center achieved a National Cancer Institute designation that comes with a $1.2 million/year grant for three years. It also expects $20 million/year in extra funding from the NCI and other sources. The NCI currently supports about $9 million/year. The Cancer Center’s research partnership with Lawrence Livermore National Laboratories (40 affiliated scientists), the first of its kind in the nation, was a key factor in winning the designation. Another factor was a massive cooperative cancer research effort created by integrating cancer investigators from programs in veterinary medicine, comparative medicine (studying similarities among humans and other animal species), biological sciences, and agriculture and engineering. The center describes itself as a “constellation of scientific expertise, focused on cancer, that doesn’t exist at other centers.” (UCD Cancer Center, 2004). A similar center for intelligence in developing and selecting foods for wellness could dovetail into existing centers to gather integrated expertise to address dietary interventions for prevention of all types of diseases. But will a Center of Centers for food and health be valuable or more confusing?

OBTAINING PRIVATE FUNDING

UC Davis has several food and health competencies that have brought private funding to support food and beverage research. One of the best known campus competencies is a 125-year-old, internationally-recognized viticulture and enology program, the first
sensory science curriculum in the world, and a Wine Business MBA in conjunction with the Bordeaux Business school in France. This program, along with food science expertise, brought $25 million from Robert and Margrit Mondavi to found the Robert Mondavi Institute for Wine and Food Science. Funds will finance facilities for the viticulture and enology and food science and technology departments and new programs.

Another competency for UC Davis is food antioxidant research. Antioxidants are believed to be important to the prevention of cardiovascular disease and cancer. UC Davis food science Professor Alloys Tappel was ahead of his time in understanding the significance and mechanism of oxidation in food and in humans. A lipid chemist, he identified Vitamin E as an important antioxidant and performed the first in vivo studies on its functions. Tappel’s pioneering research over three decades has become the mainstream of nutritional research today. Professor Ed Frankel, a graduate of the UC Davis dairy division, returned to UC Davis after a distinguished career at the USDA Peoria laboratory to collaborate with Tappel. Frankel, an analytical lipid chemist, co-authored a Lancet publication that proposed an explanation for the French Paradox (Frankel et al., 1993a). The UC Davis lipid group suspected that the lower incidence of heart disease than might be expected for the high fat French diet might be due to the antioxidant nature of red wine phenolics. In the same decade, the popularity of red wine increased from the previous decade from 17 to 40% of the wine market by 2000 (CA Assoc. of Winegrape Growers, 2002).

In April 1993, Frankel, Kinsella and Andrew Waterhouse, a professor in viticulture and enology, published a letter in the Lancet titled Inhibition of human LDL oxidation by resveratrol, the polyphenol found in grape skins and seeds (Frankel et al., 1993b). A similar level of excitement over antioxidants from chocolate began with a letter published in the Lancet in September 1996 (Waterhouse et al., 1996). Waterhouse and his graduate students analyzed cocoa powder and chocolate for total flavonoid phenolics, and compared the quantity of phenolics in hot chocolate, bakers chocolate and milk chocolate to those found in a serving of wine. They tested cocoa extract for antioxidant activity by determining its ability to inhibit oxidation of LDL cholesterol purified from human blood just as they did for wine. Among the three forms of chocolate, cocoa had the highest levels of phenols, followed by baking chocolate and milk chocolate. The conclusion was that a standard 1.5 oz milk chocolate bar had approximately the same quantity of phenols as a 5 oz glass of red wine. The article ended with the statement that “a pleasant pairing of red wine and dark chocolate could have synergistic advantages beyond their complementary tastes.”

The press release was picked up by every major newspaper in the country and kept Waterhouse in media interviews for days. The antioxidant content of chocolate has now gone mainstream. In February 2004, the Sacramento Bee contained an advertising section from California grocers Raley’s/Bel Air entitled “Something extra, A Food and Lifestyle Publication for Enhancing Quality of Life.” Opposite the wine and spirits advertised specials, it stated, “Wine chemistry professor Andrew Waterhouse of UC Davis found that chocolate and red wine contain compounds called phenolics that are thought to decrease coronary heart disease by preventing clotting of the arteries.” It featured the quotable end of the Lancet article. Since then, Waterhouse has published many additional analyses on phenolics in wine, prunes and walnuts.

If wine phenolics can prevent cardiovascular disease, what can they do for cancer? Susan Ebeler, viticulture and enology professor and chemist who studies phenolic contributions to flavor, has collaborated on research to show that wine contains compounds which can delay tumor formation in an animal carcinogenesis model. Ebeler and her lab conducted studies to identify specific compounds which may be responsible for this delay and to elucidate possible mechanisms of action.

The analytical strengths in antioxidants led to collaborations with Harold H. Schmitz, Group Manager for the Analytical and Applied Sciences Group at M&M/MARS, who sits on the College Advisory Council and serves as affiliated faculty in the UC Davis Department of Nutrition. M&M/Mars funded development of analytical methods to study polyphenolic components in chocolate. Carl Keen, chair, UC Davis nutrition department, is frequently quoted in the national publications that explain the antioxidant properties, and health benefits, of chocolate.

Communicating food and health research

How do these examples illustrate the directions for health-related research in the University environment?
The UC Davis nutrition department has more than 18 professors, and an equal number of professional researchers and adjunct professors. They study vitamins, trace minerals, fiber, obesity, longevity, infant nutrition and lactation, fat metabolism, sports nutrition and international at-risk populations. Their work is collectively more compelling to public health than chocolate research. Chocolate’s popularity, however, funded model systems and development of analytical methods that benefit antioxidant research for many other foods from California. It also brought the department national attention and established chocolate as an acceptable indulgence.

UC Davis is not unique in its ability to command the attention of the mainstream media. Michael B. Zemel from the Department of Nutrition, The University of Tennessee, Knoxville was featured in Reader’s Digest, a widely circulated US magazine, explaining that calcium intake and weight gain might be inversely related. Zemel has published eleven papers on aspects of calcium and body fat. This is one of the first publicized findings to go against the conventional wisdom and hints at what people have hoped and suspected for years, that caloric intake and activity are not the only two factors contributing to weight gain (Zemel, 2003).

Table 3. Foods analyzed for antioxidant content at UC Davis2.

<table>
<thead>
<tr>
<th>Prune</th>
<th>Beans</th>
<th>Grape</th>
<th>Tea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onion</td>
<td>Pomegranate</td>
<td>Cocoa</td>
<td>Soy</td>
</tr>
<tr>
<td>Blackberry</td>
<td>Cherry</td>
<td>Wine</td>
<td>Walnut</td>
</tr>
<tr>
<td>Peach</td>
<td>Plum</td>
<td>Fish</td>
<td>Beer</td>
</tr>
<tr>
<td>Green peppers</td>
<td>Tomatoes</td>
<td>Cloves</td>
<td></td>
</tr>
</tbody>
</table>

2CIFAR Directory of Foods for Health Research

Developing integrated disciplines

The future for food that is ‘universally approved’ for health will require that students learn new vocabularies and cross-disciplinary boundaries without sacrificing their command of basic sciences. Metabolomics, a multidisciplinary specialty that identifies and quantifies metabolites in cells, is the basis of a research concentration at UC Davis advocated by food science Professor Bruce German. Also a dairy advocate, German has brought a vision for individualized nutrition to the campus. He illustrates that what is beneficial to one person is not beneficial to the next, and that even the fittest Olympic athletes in their sport of choice do not advocate a ‘one-size-fits-all’ diet. German wrote that “a fundamental conceptual difference exists between a diseased and healthy body. Simply stated, ‘diseased’ means getting one thing wrong, while ‘healthy’ means getting everything right.” The implications of dietary fat in obesity and atherosclerosis led to a reduction in the national fat content of foods and some misleading dietary guidelines. Researchers are now discovering that fat alone will not predict chronic disease, and this has hurt the credibility of the nutrition community. German believes that the key to understanding metabolic regulation is to assess all aspects of metabolism simultaneously (German, 2002). The future will see complex systems using multiple markers to assess disease development in more defined and accurate methods. His former graduate student, Steve Watkins, Chief Scientific Officer and cofounder of Lipomics, West Sacramento, is collaborating with GlaxoSmithKline and Bayer to use its patented tools for lipid metabolite analysis. The application of this intelligence may lead to better and earlier biomarkers for diseases related to the human metabolism of lipids.

Another campus center with a vision for individual diet recommendations is the UC Davis Center for Nutrigenomics, directed by Professor Ray Rodriguez. This center is studying the influence of diet on gene expression to provide more insights on how genomics can provide optimized diets. The center is supported by a five-year, $6.5 million grant from the National Center on Minority Health and Health Disparities, a division of the National Institutes of Health. Nutritional genomics is the study of how foods interact with particular genes to increase the risk of diseases such as type 2 diabetes, obesity, heart disease and some cancers. With greater understanding of epidemiology of subpopulations and new discoveries in genomics, we may be better able to link the identification of genes with risk for disease. Some typical examples are: African-American men have a 60% higher risk of being diagnosed with prostate cancer than Caucasian men; half of all adult Pima Indians in the US have type 2 diabetes, compared to 6.5% of adult Americans of Caucasian descent. A single change in DNA in people living in Scandinavia 10,000 years ago allows most Caucasian adults today to drink milk without lactose intolerance. The center’s press release promises “Nutritional genomics will enable individuals to better manage their health and well-being by precisely matching their diets to their unique genetic makeup.” Rodriguez is a sought-after spokesperson who advocates this approach in his interviews with the media.
The role of the media and opinion leaders in communicating nutrition messages is a specialty of CIFAR board member Cheryl Toner, International Food Information Council (IFIC), Arlington, VA. IFIC has tracked consumer reactions to nutrition messages since 1998, using Cambridge-based Cogent Research. IFIC provides press kits for media and communicators on responsible reporting, tracks media coverage on emerging issues in food and nutrition, and helps opinion leaders interpret nutritional studies for the public. At the Second Annual International Nutrigenomics Conference in Amsterdam, the Netherlands, November 2003, the Cogent Syndicated Genetic Attitudes and Trends Survey was presented. “Americans are ready and willing to buy products based upon their genetic information, but the science is only in the early stages of being able to deliver,” said Christy White, principal of Cogent Research. “The good news is consumers aren’t looking for complete diet regimens, but for individual products and basic recommendations.” (International Food Information Council, 2004).

THE UNIVERSITY FORUM FOR CONTROVERSY

The university campus serves as a central focal point for airing controversy in the food industry, and has been an active participant in the debate over genetically modified foods; organic versus conventional agriculture; threats of food-borne disease; alternative food processing technologies like food irradiation; animal welfare and safety practices in meat and poultry production; and fad diets. In most of these instances, university scientists favor the technological and scientific achievements in which they are invested. Dissent against food and agricultural technologies on campus is often fostered by social sciences, history, art, literature, international, minority or gender studies. However, top-notch universities encourage constructive skepticism and dissent in every discipline.

University scientists view public backlash as a misunderstanding that can be solved through better science education. University scientists, who are typically accorded a great deal of respect in their fields, typically feel maligned and mistrusted when they participate in forums to air public controversy. Many scientists resent having to defend themselves, declining offers to participate. However, a new appreciation for the contributions of those who feel compelled to participate can be observed. At UC Davis, as at most agricultural campuses, biotechnology is central to tremendous growth in agricultural and biological education programs. Many programs were based on discoveries leading to patents or advancement of a biotechnology-based agricultural economy that promised to improve the quality of life. As biotech crops captured the attention of the media, the scientific community was unprepared for the backlash. The issues presented by activists, many based on widespread mistrust of science, politics and regulation, left scientists unprepared to address the underlying concerns. CIFAR, with a broad array of company support from small organic food processors to large multinational food and agriculture businesses, responded by organizing forums to promote constructive dialogue. Martina Newell-McGloughlin, Director, University of California Biotechnology Program, and a member of CIFAR’s executive committee, became internationally recognized as a biotechnology advocate for defending the public benefits of biotechnology in the public arena. McGloughlin agreed that the culture of science does little to promote better communication between scientists and the press. She noted in the PEW proceedings on biotechnology and the media, that “scientists who are adept at getting their position across (to a broad audience) are devalued in scientific circles as populists or as exaggerating.” She cited the example of the late Carl Sagan, a scientist who was enormously popular with the mass media but who was “never elected to the National Academy of Sciences.” (McGloughlin, 2002). Others point out that faculty do not get grants, tenure, or advance professionally by talking to the media. While this may be true for food controversy, when they bring good news to the forefront that concerns a food to improve health, the results speak for themselves.

Five years later, after the events of 9-11 allowed the genetically engineered food backlash to cool on a back burner, campus factions agreed to disagree, validated by a new appreciation for values inherent in the marketplace. Participation in the debate gave stakeholders a new appreciation for the importance of communication and transparency in the implementation of new technology. The dual techniques of conventional breeding and recombinant bioengineering are likely to continue in parallel as long as the public demands alternatives. In the food science department, award-winning toxicologist and public educator Carl Winter warns consumers about the high rates of food-borne illness from organic
produce (IFT, 2002) while down the hall, environmental toxicologist Alyson Mitchell compares the antioxidant content of conventionally and organically grown fruits and vegetables, reporting data that sometimes favor organic methods. The college initiative in foods for wellness has invigorated food and agricultural research by providing a common goal, regardless of technology. The food biotechnology controversy illustrates that a university cannot afford to be single-minded in its approach to problems and maintaining the traditions of academic freedom ensures that it will not.

CIFAR continues to help prepare the campus for emerging controversy. A collaboration with trends forecasting partner Nuffer, Smith, Tucker, San Diego, (NST) provides opportunities for faculty to sit on an international panel of leaders in food and agriculture to discuss issues as they emerge. The CIFAR board benefits from this process through NST summaries that are presented at the biannual board meetings and respond to the emerging issues through their support of communications strategies, or research sponsorship.

TEACHING FOOD, CULTURE AND LIFE

An important aspect of CIFAR conferences is offering the scientific community an appreciation for culinary arts. Conferences in Napa Valley at the Culinary Institute of America and Copia have featured speakers that keep scientists mindful of the taste and flavors that rule the marketplace. The Research Chef’s Association (RCA) has inspired food science students to broaden their educational horizons to aspects of menu development as well as science in their education. UC Davis does not offer degrees pertaining to the hospitality industry, however campus programs welcome chefs and food service professionals to learn about food and nutrition science through short courses and conferences on food, health and agriculture. This spring we hosted our second year of continuing education courses dedicated to chocolate in the food science department. CIFAR also serves as a central resource for International collaborations. Director Sharon Shoemaker is visiting professor at three Chinese universities and joins the Food Science and Technology department in co-hosting a joint conference each other year with Southern Yangtze University in Wuxi, China, the leading school of food science in China. Each CIFAR conference typically features a presentation on research pertaining to ingredients typical of Chinese medicine. Shoemaker also secures funding for students to attend international conferences. CIFAR support has come from China, Thailand, Denmark, Britain, Japan, and Finland.

Conclusion

Food is an important factor in every aspect of our life. Our physical and mental health, culture and rituals depend on food’s appeal, affordability, and its ability to comfort and nurture. The nation’s headlines illustrate the complexity of the systems inherent in providing the wide variety of affordable foods we now enjoy. The future promises to keep this generation living longer to enjoy foods on more occasions than ever before possible. The next generation of food scientists must feed a large, mobile population in an interdependent global economy and a changing environmental climate. Today’s students are entrusted with the protection of the food supply from toxic contamination and new sources of disease. And if our food traditions and cultural preferences are equally important, students must learn to use emerging technologies in ways that keep foods as appealing as the foods they remember from childhood. UC Davis, situated on the Pacific Rim, will continue to be a leading innovator in foods for wellness, both in education and research. The vision for foods for health and wellness that captures the synergistic value of all the inputs central to delivering benefits to the student, the public and the economy, is still coming into focus as one university searches for intelligence in a small universe of foods for health.

References


How does diet influence health – could the food chain benefit from a more proactive approach to clinical nutrition issues?

JOHN C. MACRAE

Rowett Research Institute, Bucksburn, Aberdeen, United Kingdom

Over recent years, in the UK and Europe the food chain and particularly its animal produce suppliers, have been frequently challenged by clinicians and public health authorities with health scares, such as salmonella in eggs, E. coli in meats, BSE and FMD. These have, in some cases, had dramatic effects on consumer choice, leading to major economic losses in the sectors supplying the produce. Since 1999 the Rowett Institute, which has a long history of nutrition research on farm animals, has re-focused its research into the area of diet and (human) health, with programmes that are attempting to elucidate how specific nutrients can help to offset the incidence of human non-communicable diseases, such as coronary heart disease (CHD), colon cancer and obesity. It would seem to us that some important information is beginning to emerge from these biomedical programmes that could be used by the animal sector of the food chain as specific ‘health benefit’ messages in promoting animal products in terms of their ability to help prevent some of the non-communicable diseases.

There is a vast literature linking, either through epidemiology or within more mechanistic studies, the inappropriate consumption of specific nutrients with the incidence of CHD (Krauss et al., 1996; Hu et al., 2001) and cancer (Wynder et al., 1997; Department of Health, 1998). Consideration of some of this may provide a basis for future strategies within the animal sector of the feed industry.

Fats

SATURATED AND POLYUNSATURATED FATS

From the human health perspective, there has been a history of concern about the lipid components of foodstuffs, with ‘animal fats’ (especially ruminant saturated fats) being viewed with particular suspicion. Indeed, it is possible to trace a timeline of awareness about the problems and benefits of animal fats and their association with health problems over the last 30 years. Starting back in the 1960/70s epidemiologists identified strong relationships between the proportion of the dietary calories consumed as saturated fat and the incidence of CHD and colon, prostate and breast cancer. The CHD story related to the influence of saturated fat on the development of high levels of cholesterol in the circulating serum lipoproteins, with polyunsaturated fats (PUFA) seemingly modulating these rises in risk (Hegsted et al., 1965). This led to recommendations for a reduction of saturated fat in the diet. Consumers were not slow to take up this message, as witnessed by a major reduction in the full-fat liquid milk sales in the UK between 1984 (90% of total) and 1997 (<25% of total).

OMEGA-3 POLYUNSATURATED FATTY ACIDS

More recently, in the early 1990s research started to show that the different PUFA were not all equally beneficial in terms of preventing the onset of the non-communicable disease. Inflammatory responses are important components in the development of CHD and cancer and immunologists identified that the omega-6 (n-6) PUFA (e.g. linoleic acid, C18:2) are less beneficial than the omega-3 (n-3) PUFA (e.g. linolenic acid, C18:3; eicosapentaenoic acid, C20:5 (EPA); docosahexanoic acid, C22:6 (DHA); Gibney and Hunter 1993). This relates to the pro-inflammatory eicosanoids generated during the post-absorptive human metabolism of the n-6 fatty acids (FA). The n-3 FA have the ability to modulate
this inflammation by competing with the n-6 metabolites for incorporation into immune cell membrane phospholipids. Present recommendations are to increase the intake of n-3 FA towards a dietary optimum n-3:n-6 FA ratio of 0.4-0.5. However, most human foodstuffs have a ratio nearer to 0.1-0.2, hence the health benefits of fish oil products (Leaf et al., 2003), which contain high levels of n-3 FA (see Figure 1).

The reason why fish oils are an abundant source of the longer-chain n-3 FA relates to the high linolenic content of chloroplast lipids. The presence of chloroplasts in marine phytoplankton is the basic building block of the Marine Food Web. They are also the crucial component of fresh forages and so, not surprisingly, recent research has started to identify substantial increases in the n-3:n-6 PUFA ratio in the lipids of animals fed fresh forages rather than concentrates (see Dewhurst et al., 2003). Advocates of the current drive for sustainability may see this as an important longer-term positive (and specific) ‘health’ message to help promote the sales of pasture-reared beef and sheep and the milk from forage-fed dairy cows. However, linseed oil also has a very high content of (n-3) linolenic acid (see Figure 1) and could be an important consideration in the processing of concentrates for the animal industry.

CONJUGATED LINOLEIC ACIDS

If the n-3 PUFA were the human nutrition interest of the early 1990s, over the last five years equal, if not more, attention has been directed to the conjugated linoleic acids (CLA). A plethora of laboratory animal studies has reported apparent health benefits from the consumption of CLA. These have included reducing the severity of cholesterol-induced aortic lesions in rabbits (Lee et al., 1994), reducing the incidence of carcinogen-induced mammary tumours in mice (Ip et al., 2001) and even altering the composition of body weight gain (higher protein/lower fat) in mice (Park et al., 1997). Recent studies at the Rowett would suggest that at least in the CHD area, one of the main mechanistic attributes of the CLA is their ability to modulate the inflammatory mechanisms at the level of adhesion molecule transcript in endothelial cells. The transcription of these adhesion molecules, which lead on to plaque formation, is thought to be stimulated by cytokines.

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**Figure 1.** Relative concentrations of the longer-chain fatty acids in meat, milk, plant oils and fish oils and the basis of the high n-3 FA content of fish oils.
generated from the oxidation of low-density lipoproteins (LDL) in the tissue that underlies the endothelial cells. The CLA seem to modulate these cytokine signals and so reduce the inflammatory response (Cook et al., 1999) in a not dissimilar way to the n-3 PUFA.

The CLA are very much an attribute of ruminant products, being formed as an intermediary metabolite in the biohydrogenation of C18:2 (unsaturated) linoleic acid to 18:0 (saturated) stearic acid during rumen fermentation. Hence, levels of CLA in milk, cheese, butter, lamb and beef (4-7 mg/g total FA) are considerably higher than in non-ruminant products (chicken, pork, fish, olives; <1mg/g). Unfortunately, these levels could never provide sufficient CLA intake per day to make any meaningful contribution in terms of offsetting inflammation in humans; at present a person would need to consume over 3.5 kg of cheese per day to take in a meaningful therapeutic dose of CLA. Currently, attention is being focused on the regulation of biohydrogenation in rumen microbes, either by dietary manipulations (Loch and Bauman, 2003), or by examination of those microbes that regulate the latter steps in this process and whether these could be manipulated to boost the CLA content of muscle and milk products (John Wallace, personal communication). If this could be achieved, then it may represent another specific health benefit with which to make ruminant products more attractive to the consumer.

**Selenium**

One of the underlying mechanisms associated with the onset of CHD and cancer is disruption of the normal cellular processes. In this respect, lipid oxidation is a major stressor, because the cytotoxic hydroperoxides formed can cause membrane damage. Intracellular free radical generation, accelerated in exercise, infection and even the stress of high performance, is another stressor. To modulate these processes cells depend on antioxidants such as vitamin E and a number of glutathione peroxidases (GSH-Px) (Figure 2). The GSH-Px are selenoproteins, and to date 25 have been identified in the human genome. It is not surprising therefore that early epidemiology studies indicated clear links between the availability of selenium (Se) in the human diet (Se levels in blood) and the incidence of CHD (Salonen, 1982) and cancer (Clark, 1985). Some of the selenoproteins are important also in thyroid metabolism and in the redox control of cells; and so inadequate selenium intake has been linked with impaired thyroid metabolism, reduced response to viral infection, infertility and, in more serious situations, cardio- and skeletal-myopathies.

One concern for clinicians in the UK and other parts of Europe over the last 10-15 years has been the substantial reduction in daily Se intake that has

![Figure 2. The role of selenium (glutathione peroxidases) in the antioxidant function of cells.](image-url)
occurred as a result of the switch from selenium-rich high-protein North American wheat to lower-Se UK and European wheat for flour making in the mid 1980s. The volcanic and sandy soils across major sectors of the UK have had a lot of their Se washed out, leading to low levels of Se in cereals, fruit and vegetables; and as a result, the daily intake of Se in the UK population (~35 µg/d) is less than half of that in the USA (80-100 µg/d). The question of whether the UK should follow the example of Finland and New Zealand and add sodium selenate to fertilisers, or Se to bread flour was raised in the late 1990s (Rayman, 1997), but as yet nothing has been done.

Selenium deficiency in farm animals has been well recognised for many years. In cattle, severe deficiency will result in myodegenerative problems such as white muscle disease, whilst marginal deficiencies have been linked to elevated levels of mastitis, scours, cystic ovaries and retained placenta (Villar et al., 2002). As a result Se has been included in most mineral supplements for farm livestock since 1978 and therefore the Se content of animal products (e.g. meat and poultry: 100 and 160 µg/kg respectively; FSA, 2002) and particularly liver and kidney (>400 µg/kg), are considerably higher than in the plant components of the UK diet (fruit and vegetables, <10 µg/kg; cereals, 20-25 µg/kg; and bread made from UK and European wheats, 50-55 µg/kg; see FSA, 2002). It has been estimated that animal products presently provide over 60% (18 µg per day) of this intake. The present reluctance of governments to implement supplementation policies lies in the potential toxicity problems associated with over-consumption of selenium (>900 µg per day). However, there seems no danger of reaching these dangerous levels by advocating the promotion of ‘health-enhancing’ animal products, (alongside the odd Brazil nut!) to help replete the marginal selenium intakes that have developed over the last twenty years.

Value-added aspects of animal proteins

Whilst the nutritive value of animal proteins is recognised to be higher than that of plant proteins, (FAO, 1970), they are also more expensive. The extra costs of production relate predominantly to the inherent inefficiencies associated with conversion of dietary (plant) proteins into animal products. Even in chickens and pigs, net protein deposition represents only 30-40% of the consumed dietary protein, but in ruminants the figures are much lower, 25-30% in dairy cows but only 15-20% in growing beef and sheep. Therefore, in terms of presentation of these products to the health-conscious, but also cost-conscious consumer, it may be important to consider certain value-added aspects associated with the consumption of milk and meat proteins.

MILK PROTEINS

These comprise two distinctly different types, whey proteins (20%) and caseins (80%). There are several different types of whey protein (the protein fraction which associates with the whey fraction during cheese-making), each with its own particular importance. β-Lactoglobulin, for example, which comprises about half of the total whey protein, is a retinol-binding protein essential for vitamin A absorption. It also has a high content of cysteine, which can help bolster glutathione production (given adequate Se availability; see above). However, it is the casein fraction that makes milk also a ready source of calcium, phosphorus and magnesium.

This property was identified many years ago (Orr, 1928) and subsequently led to the provision of free milk to school children in the UK in order to alleviate the crippling disease of rickets and accelerate growth; it also provided a much needed market for this agricultural product. Recent estimates have suggested that milk and dairy products supply half of the calcium intake for most individuals in the UK plus 25-30% of the daily requirement of the B vitamins. Milk is also a ready source of vitamin A, D, E and K, but these are all fat-soluble and so their provision from milk has been reduced over recent years as public perception of the health risks of saturated fat has led to more and more consumers switching to semi-skimmed (2% fat) and skimmed milk.

MEAT PROTEIN

In the same way that milk protein can provide a vehicle for the delivery of calcium and phosphorus, meat can provide a ready source of a number of trace elements, including iron. Considerable clinical attention has been focused recently on the problems associated with low iron intake in pregnancy, when as many as one in five women are clinically iron deficient and many more suffer from marginal iron deficiency. If this anaemia is not corrected there is an increased risk of poor pregnancy outcome, premature delivery and/or low birth weight. In
addition, the child has a higher risk of developing cardiovascular disease or non-insulin dependent diabetes later in life. Consequently, pregnant women with anaemia are always subscribed iron supplements. However, these can cause unpleasant side effects (e.g. gastric upset, nausea and constipation) and so compliance rates are low.

The basic problem with inorganic iron supplements is one of bioavailability. Less than 10% of ingested inorganic iron, or indeed, the non-heme iron of most plant foodstuffs, is absorbed from the gastrointestinal tract. In comparison, heme iron, (i.e. the iron bound up in the porphyrin ring structures of haemoglobin and myoglobin (see Figure 3), present in meat and fish, has a much higher bioavailability (20-30%, Roughead and Hunt, 2000). Given the clinical problems referred to above and the experimental information from rodent models, linking iron deficiency in pregnancy and the subsequent development of hypertension problems in the growing offspring (Gambling et al., 2003), there would appear to be considerable potential for promoting meat as a means of alleviating anaemia, not just during pregnancy, but through other periods of high iron requirement such as adolescence and menstruation. This could be helped by the fact that the iron-carrying capacity of different qualities of meat seems to be linked to eating quality as perceived by taste panel assessment; a relationship built on the fact that
differentials in meat tenderness can be correlated with differences in the area and frequency of the slow twitch oxidative fibre in the muscle (Maltin et al., 2001) with higher myoglobin content.

Meat is also a major dietary source of other micronutrients such as zinc, selenium and copper and the vitamins folic acid and B12; indeed B12 is only found in animal and fish products. Each of these nutrients has been linked to the prevention of non-communicable diseases and to the maintenance of a healthy immune system and so it is perhaps time for the animal industry to start to consider the products that it delivers to market not just in terms of consumer (organoleptic) preference, but also in terms of specific health benefits.

**Conclusions**

This paper has attempted to draw on biomedical information that might be used by the animal industries in the promotion of specific health messages to support niche markets. For example, the omega-3 content of meat and milk products from pasture-fed ruminants and the selenium and iron carrying capacity of meat products, particularly as the latter seems to be enhanced in quality breeds such as the Scottish Aberdeen Angus. In the future, particularly if the microbiologists can find ways of

![Figure 3. Comparative bioavailability of haeme and non-haeme iron.](image-url)
controlling biohydrogenation in the rumen, the CLA content of ruminant products could also make a powerful ‘healthy eating’ message. However, promotion of such messages may need a refocus of research effort. Over the last five years there have been more than 1000 research papers from studies that focused on the tenderness, texture, juiciness and flavour of meat products. Perhaps, as we go through the next five years more thought should be given to the nutritive value of the products placed on the market. Indeed, increasingly we could be faced with the realisation that ‘healthy products’ are the essence of an (economically) healthy industry.

References


Functional components of the cell wall of *Saccharomyces cerevisiae*: applications for yeast glucan and mannan

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**Introduction**

In recent years, there has been increasing biotechnological and commercial interest in yeast cell wall components, including their use as biological response modifiers, anti-cancer agents, bioadsorbents, ingredients in food processing and cosmetic formulations, and as systems for immobilizing oral vaccines, antibodies and enzymes of industrial significance (Fleet, 1999). This increase in interest has occurred concomitantly with our better understanding of the chemical composition, genetic regulation and functional properties of cell wall components.

Integrity of the cell wall of *Saccharomyces cerevisiae* is required for cell viability under various environmental conditions. The principle functions of the wall are to prevent lysis under hyper- and hypo-osmolarity conditions, provide shape for the cell, form a permeability barrier for macromolecules, and participate in cell-cell recognition (Orlean, 1997; Estrem and Skatrud, 2001). In addition, it provides a matrix for a variety of enzymes involved in hydrolytic processes, nutritional uptake, end-metabolite secretion and cellular maintenance. The cell wall is a dynamic structure that can adapt to physiological changes (i.e. from logarithmic to stationary phase), morphological changes (conjugation, sporulation or pseudohyphal growth) or environmental conditions (nutrient and oxygen availability, temperature and pH) (Orlean, 1997; Kapteyn *et al.*, 1999; Aguilar-Uscanga and François, 2003).

The universal adoption of the yeast cell wall and its individual components, mannan oligosaccharides and β-glucan, as functional ingredients in animal feed has not been transferred to the human food industry. A review of the current state of knowledge with relation to yeast cell wall components will emphasize areas where they could be used as functional constituents of human food products. Central to functionality of a food ingredient is its structure and composition, which will be briefly discussed.

**Cell wall composition of *Saccharomyces cerevisiae***

The cell wall comprises 15-30% of the dry weight of the cell with the major components being β(1,3) glucan, β(1,6) glucan, mannoproteins and chitin (Table 1). These components are covalently linked to form macromolecular complexes, which are assembled to form the intact wall, often likened to a ‘flexible building block’ (Kollar *et al.*, 1997). In reality, due to the cell wall components only occupying 10-20% of the wall volume, a better analogy is that the wall is comparable to a latticework, rather than solid structure (Figure 1) (Lipke and Ovalle, 1998). Arrangement of the cell wall is based on an internal skeletal layer consisting of β(1,3) glucan molecules that form a three-dimensional network surrounding the entire cell. This network is held together by local alignments between segments of β(1,3) glucan molecules, allowing the formation of multiple hydrogen bridges. External to the skeletal layer, cell wall mannoproteins are linked to the non-reducing ends of β(1,3) glucan chains either directly (Pir-CWPs) or indirectly through an interconnecting β(1,6) glucan moiety (GPI-CWPs). Following cytokinesis, the skeletal layer becomes fortified by coupling of chitin chains to non-reducing ends of β(1,3) glucan chains. These chitin chains are
found close to the plasma membrane (Kollar et al., 1997).

### Table 1. Major components of S. cerevisiae cell walls (adapted from Lipke and Ovalle, 1998).

<table>
<thead>
<tr>
<th>Component</th>
<th>Mean molecular mass (kDa)</th>
<th>% of cell mass</th>
<th>Relative molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$(1,3) glucan</td>
<td>240</td>
<td>30-50</td>
<td>1.0</td>
</tr>
<tr>
<td>$\beta$(1,6) glucan</td>
<td>24</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Mannoprotein</td>
<td>100-200</td>
<td>25-50</td>
<td>1.2-2.4</td>
</tr>
<tr>
<td>Chitin</td>
<td>25</td>
<td>1-3</td>
<td>0.1-0.3</td>
</tr>
</tbody>
</table>

β-Glucans are generally described as polymers of glucose and form the major structural polymer in the cell wall and encompass the entire cell with a microfibrillar net (Kollar et al., 1997; Lipke and Ovalle, 1998). Three classes of β-glucan have been described: a) alkali insoluble-acetic acid insoluble β(1,3) glucan, b) alkali soluble (β-1,3) glucan, and c) highly branched β(1,6) glucan (Fleet, 1991; Smits et al., 1999). The alkali insoluble-acetic acid insoluble β(1,3) glucan is believed to be involved in maintaining wall mechanical strength and shape; and the alkali soluble β(1,3) glucan has been proposed to confer flexibility in the cell wall. β(1,6) glucan plays a central role in cell wall organization, interconnecting with the β(1,3) glucan, mannoprotein and chitin (Kollar et al., 1997). Structural studies, using solid-state nuclear magnetic resonance on intact yeast cells, have determined β(1,3) glucan to possess a helical confirmation (Krainer et al., 1994). Such helices are composed of a single polysaccharide chain or of three hydrogen-bonded chains (a triple helix) (Figure 2).

Yeast wall mannoproteins are highly glycosylated polypeptides, often 50-95% carbohydrate by weight, that form radially extending fibrillae at the outside of the cell wall (Lipke and Ovalle, 1998; Kapteyn et al., 1999). Many mannoproteins carry N-linked glycans with a core structure of Man$_{10-14}$GlcNAc$_2$-Asn structures very similar to mammalian high mannose N-glycan chains. ‘Outer chains’ present on N-glycans consist of 50-200 additional α-linked mannose units, with a long α(1,6)-linked backbone decorated with short α(1,2) and α(1,3)-linked side chains. Until recently the identification of proteins in the cell wall has been hampered by the complex nature of the cell wall structure and its relative resistance to simple digestion and extraction. A novel method was developed to tag and identify cell surface proteins using a method based on treating intact cells with a membrane-impermeable biotinylation reagent that specifically reacts with free amino groups (Casanova et al., 1992). Using this method, the

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**Figure 1.** Structural components of the cell wall of *S. cerevisiae* (adapted Lipke and Ovalle, 1998).
identity of approximately 20 cell wall-associated proteins was confirmed (Mrsa et al., 1997), although following a genomic approach greater than 40 have been predicted (Smits et al., 1999). Two distinct classes of cell wall proteins can be distinguished, GPI (glycosylphosphatidylinositol) proteins and PIR (proteins with internal repeats) proteins (Kapteyn et al., 1999). The GPI proteins are linked to other cell wall components through a remnant of their GPI anchor and β(1,3) glucan crosslinks the proteins to β(1,3) glucan. An example is the α-agglutinin protein. The PIR proteins are less well understood, but in contrast to the GPI proteins, are not posttranslationally modified by addition of a GPI anchor, but are highly O-glycosylated (Mrsa and Tanner, 1999). The mannoproteins determine the surface of the yeast cell and are responsible for the cell’s antigenic behavior.

Chitin, quantitatively a minor component of the cell wall, is glycosidically linked to non-reducing branches of the β(1,3) glucan and β(1,6) glucan (Kollar et al., 1997). Addition of chitin to β(1,3) glucan structural units is essential for the insolubility of the cell wall, by transforming alkali soluble material into an alkali insoluble state. The structure of α-chitin is similar to α-cellulose, with hydrogen-bonded anti-parallel chains of N-acetylglucosamine units (Lipke and Ovalle, 1998). Hydrogen bonds involving the amide groups (absent in cellulose) further stabilize the crystals. Due to its low concentration in yeast, chitin has not been commercially exploited.

Yeast glucans in food and health

GLUCANS AND IMMUNE RESPONSE

Hot water extracts from various mushrooms and tree fungi have been used for centuries as folk remedies in Japan, China and Eastern Russia for cancer therapy or their general health stimulating properties (Kogan, 2000). During the 1940s in Europe, a crude yeast cell wall extract (from *S. cerevisiae*) called Zymosan was investigated for its purported drug-like activities. Research conducted by Louis Pillemer and his colleagues demonstrated that Zymosan could nonspecifically potentiate and modulate the immune system, regardless of the type of invader or pathogen (Fitzpatrick and DiCarlo, 1964). Extensive research in the 1960s related the biological activity to the presence of the polysaccharides belonging to the class of β-glucans having the common structure of α(1,3) linked backbone with the single glucosyl units attached to the backbone through (1,6) glycosidic linkages (DiLuzio, 1983). Since these early discoveries there have been hundreds of papers and patents describing the properties of fungal β(1,3) glucans. Yeast β(1,3) glucans belong to a class of drugs known as ‘biological response modifiers’ (BRMs), which modify the host’s biological response by stimulation of the immune system (Sandula et al., 1995). By stimulating the host’s defense mechanisms against disease challenge rather than attacking the infective or tumor-causing agent, β(1,3) glucan remains non-toxic to the cells of the host organism. This is often not the case with synthetic or semi-synthetic therapeutics.

The main immunopharmacological activities of β-glucan include: increase of the host’s resistance to viral, bacterial, fungal and parasitic infections, an anti-tumor effect and prevention of carcinogenesis, radioprotectivity and an adjuvant effect (DiLuzio, 1983; Bohn and BeMiller, 1995). The protective action of β(1,3) glucans has been described as nonspecific immunomodulation due to involving a number of different immune pathways including macrophage activation, T-cell stimulation, stimulation of reticulo-endothelial system (RES), activation of natural killer (NK) cells, activation of the classical and alternative complement pathways and increased antibody production. Among these, macrophages are the best characterized target for β(1,3) glucans (Czop, 1986). Activation of macrophages with glucan increases their size and...
number, stimulates secretion of lysozyme and TNF, and increases the phagocytosis of antigens (Meira et al., 1996). Macrophage activation is mediated via toll-like receptor 2 (TLR2) (Underhill et al., 1999; Pivarcz et al., 2003). Upon stimulation with glucan, TLR2 is recruited to the phagosome and signals the production of TNF-α through the NF-κB pathway. TLR2 cooperates with the CD14 receptor in response to glucan, as suggested by the high levels of NF-κB observed in TLR2+/CD14+ macrophages exposed to the ligand compared to the levels obtained in TLR2+/CD14- macrophages. Furthermore, TLR6 and TLR2 were reported to coordinate macrophage activation by glucan (Ozinsky et al., 2000).

GLUCANS AND MYCOTOXIN ADSORPTION

Mycotoxins are secondary metabolites produced by fungi of various genera before or after harvest, during transportation or during storage. Some of the agricultural commodities affected are cereal grains, soybeans, peanuts and forage crops. It has been estimated that there are at least 300 mycotoxins known to induce signs of toxicity in mammalian and avian species, with more being discovered as our analytical capabilities develop (Devegowda et al., 1998). The most significant mycotoxins in naturally contaminated foods and feeds are aflatoxins, ochratoxins, zearalenone, T-2 toxin, vomitoxin and the fumonisins. Biochemically diverse with many pharmacological effects, these toxins can have a deleterious impact on animal health at extremely low concentrations.

Various strategies have been employed to control the effects of mycotoxin contamination, including mycotoxin adsorption to nutritionally inert sorbents to decrease bioavailability (Piva and Galvano, 1999). The formation of a sorbent-mycotoxin complex reduces the availability of the toxin to be absorbed across the gut epithelium and results in the eventual excretion of the toxin. Numerous inorganic sorbent materials have been tested including hydrated sodium calcium alumino-silicate (HSCAS), zeolites, bentonites, clays and activated carbons. There are a number of problems associated with their inclusion in the diet, namely high inclusion levels, narrow range of adsorption efficiency, select number of toxins absorbed, and risk of dioxin contamination of clay materials (Devegowda et al., 1998). In recent years, research into the generation of biological products to reduce the bioavailability of mycotoxins has gained momentum. Early investigations of a live yeast culture (Yea-Sacc\textsuperscript{1026}, Alltech Inc.) to suppress the effects of aflatoxicosis in poultry found an improvement in hatchability (McDaniel, 1991), improvement in broiler body weight gain (Stanley et al., 1993; Devegowda et al., 1995) and an enhancement in the immune response (Devegowda et al., 1995). In vitro studies have confirmed the dose-dependent binding of aflatoxin to purified yeast cell wall (Devegowda et al., 1994). Other in vitro studies have demonstrated that the cell wall of S. cerevisiae is capable of binding a wide range of toxins; including zearalenone (66.7%), fumonisin (67.0%), DON (12.6%), ochratoxin (12.5%), citrinin (18.4%), T-2 toxin (33.4%), DAS (12.7%) (adapted from Devegowda et al., 1998).

Dawson and co-workers (2001) have investigated the use of adsorption isotherms to further understand the mycotoxin-cell wall fraction interactions, including the adsorption affinity (K\textsubscript{s}), saturation point (K\textsubscript{sat}) and absorption capacity (Q\textsubscript{max}) of the material. The use of adsorption isotherms recognizes the fact that mycotoxin adsorption in biological systems is a reversible process that can be characterized as a chemical equilibrium. Consequently, adsorption is a concentration-dependent process influenced by mycotoxin concentration, the amount of adsorbent, and the relative affinity of the adsorbent for the mycotoxin. Moreover, adsorption isotherms can be used to compare the relative binding capacity and affinity of different adsorbents. Using this method, Dawson and co-workers (2001), compared the efficiency of aflatoxin B\textsubscript{1} adsorption by a yeast cell wall preparation (Mycosorb\textsuperscript{®}, Alltech, Inc.) to two commercial mycotoxin clay-based adsorbents (Figure 3). Mycosorb\textsuperscript{®} was demonstrated to be a more effective adsorbent at low aflatoxin concentrations, had a higher affinity for aflatoxin and possessed an overall greater capacity for the toxin. Researchers in France have recently proposed an alternative ligand-toxin model based on Hill’s equation (Yiannikouris et al., 2003a). They modeled data from an experiment studying the interaction between zearalenone and yeast cell walls and found an improved fit of the experimental data compared with an isothermal saturation curve. Furthermore, they found that the new model provided clues for the biological interpretation of toxin-cell wall interaction, an interpretation not possible using an adsorption isotherm model. The data showed that interaction between zearalenone and the cell wall is co-operative, supporting the hypothesis that the three-dimensional mobility of yeast cell wall is probably
important in the adsorption event. This finding is in contrast to that found with inorganic binders that display isothermal behavior (not co-operative), which can be ascribed to their rigid structure (Grant and Phillips, 1998; Yiannikouris et al., 2003a). In a separate study, Hill’s equation was used to compare zearalenone adsorption by four yeasts differing in cell wall composition (glucans, mannans and chitin) (Yiannikouris et al., 2003b). Additionally, the four yeasts were fractionated to prepare three components (total cell wall, alkali-soluble glucan, alkali-insoluble glucan) to aid in the elucidation of the adsorption process and identify the yeast cell wall component involved. The results clearly demonstrated that there were differences between yeast strains in their capacity to adsorb zearalenone and that the glucan concentration in cell wall can explain 83.6% of the adsorption. The sigmoid shape of adsorption curves, analyzed according to Hill’s model, indicated a cooperative association existing between zearalenone and yeast cell wall at low concentrations. Interestingly, there was a negative correlation between chitin content in the cell wall and the adsorptive capacity of glucan, further suggesting that the three-dimensional conformation is important in the adsorption phenomenon. Future work is needed to evaluate the adsorption of mycotoxins other than zearalenone by yeast glucan and further elucidate the physiochemical mechanisms involved in the mycotoxin-glucan interaction.

The mycotoxin adsorption properties of yeast cell wall and its derivatives have been confirmed in numerous in vivo studies involving poultry (Swamy and Devegowda, 1998; Raju and Devegowda, 2000; Swamy et al., 2002a), swine (Trenholm et al., 1994; Swamy et al., 2002b; Swamy et al., 2003) and cattle (Whitlow et al., 2000; Akay et al., 2003).

**GLUCANS AND CHOLESTEROL**

Heart disease continues to be the leading cause of death in the US and developed world. Increasingly, medical experts advocate dietary changes to reduce the risk of developing the disease. Ingestion of soluble β-glucan has been shown to improve the pattern of lipids in humans and experimental animals with elevated serum cholesterol (hypercholesterolemia) (Bell et al., 1999). In clinical studies, the reversal of hypercholesterolemia has been demonstrated with dietary supplementation of oats or oat bran and purified β-glucans from yeast. Oat β-glucans are found in various breakfast cereals and snacks, and their promotion as a natural functional food with the approval of the FDA has become a big business for the breakfast food industry. However, several servings
of oat-based foods containing greater than 0.75 g (per serving) of β-glucan are required to obtain the amount claimed to reduce the risk of heart disease (Bell et al., 1999). A more concentrated form of β-glucan (>74%) can be obtained by extraction from the yeast cell wall and has the potential to be added to a greater variety of everyday foods or beverages as a nutraceutical.

In a ground-breaking study, the effect of yeast β-glucan on blood serum lipids in 15 obese hyper-cholesterolemic (>240 mg/dL) men was evaluated (Nicolosi et al., 1999). After a 3-week period in which subjects ate their usual diet and their baseline blood cholesterol levels were determined, all men received 7.5 g of β-glucan dissolved in orange juice twice daily for eight weeks. Blood analysis was conducted at weeks 7 and 8, and again at week 12 (4 weeks post-treatment). The results of the study demonstrated that supplementation of the diet with 15 g β-glucan from yeast per day significantly lowered total and LDL cholesterol and improved HDL cholesterol by 16%. Further work needs to be conducted to optimize β-glucan dose and investigate long-term effects of supplementation on blood lipid chemistry. The eventual goal would combine β-glucan supplementation with a special diet to reduce or eliminate the necessity for cholesterol-lowering drugs (Nicolosi et al., 1999).

**GLUCANS AS FUNCTIONAL INGREDIENTS**

Amid the competitiveness of the food manufacturing industry, the manufacturer is always looking to develop new ingredients to decrease the cost of raw materials. The modern health conscious consumer seeks natural and healthy foods. In stark contrast, it is enough to look at the ingredients list on the label of most processed foods and see that today’s food reads like the Merck Chemical A-Z Index. This does not mean that the food is necessarily harmful, however the consumer often perceives it that way. These chemical ingredients are often added to support the physical properties of the food. Functional properties such as fat binding capacity, emulsion capacity and control of foaming are of major importance in the production of certain processed foods (Romero and Gomez-Basauri, 2003). The use of natural ingredients is often difficult to justify based on high cost or high inclusion being needed, which may have a negative impact on the final product. Yeast β-glucan is one ingredient that has demonstrated potential in improving the physical properties of food products; being used as a thickening, water-holding, emulsifying stabilizer or oil-binding agent (Thammakiti et al., 2004).

**Mannan oligosaccharides and health**

**RESPONSES IN ANIMALS**

Mannan oligosaccharides (MOS), in the form of Bio-Mos® (Alltech Inc.) have been shown to be effective in improving the health and performance in a variety of species. The use of Bio-Mos® as an alternative to antibiotic growth promoters in calf milk replacer was first studied in the early 1990s (Newman et al., 1993; Jacques and Newman, 1994). These investigators observed a reduction in fecal coliforms, an improvement in weight gain and early dry feed intake. In addition, the incidence of respiratory infection was greatly reduced in calves receiving Bio-Mos®. These findings sparked a great number of trials in different species to elucidate the mode of action and evaluate the effect of MOS as a feed additive. Recently, a series of independent meta-analyses on the data gathered from global research studies over a 10 year period have been conducted to investigate the effect of dietary inclusion of Bio-Mos® on the performance of nursery pigs (Pettigrew, 2000); broilers (Hooge, 2004a) and turkeys (Hooge, 2004b). Meta-analysis is a powerful statistical tool that allows researchers to compare results with a large database of different trials involving a particular product. With each meta-analysis conducted, the reviewers arrived at the same conclusion - Bio-Mos® improves growth performance and should be recommended for inclusion in the respective diet. The meta-analysis with weaning pigs, involving 55 comparisons (29 separate experiments and 21 research teams) demonstrated a 4.15% improvement in weight gain, 2.34% improvement in feed conversion and 2.08% increased feed consumption (Pettigrew, 2000). In the broiler meta-analysis, the antibiotic control and Bio-Mos® diets gave statistically equivalent performance with regard to growth promotion and feed utilization (Hooge 2004a). However, Bio-Mos® diets resulted in -17.2% relative change in mortality averaging by treatment and -18.1% averaging by trial compared to antibiotic controls. This indicated that Bio-Mos® improved (P<0.01) broiler livability compared to the antibiotics evaluated (including avilamycin, bacitracin, bambermycin or virginiamycin at growth promoting concentrations). Data in turkeys are similar to the
findings in broilers; a meta-analysis involving 27 comparisons of turkeys fed Bio-Mos® versus antibiotic-free control diets demonstrated that on average Bio-Mos® improved body weight (+2.09%, P=0.01) and FCR (-1.47%, P=0.17) and mortality (P=0.016) relative to the non-medicated control (Hooge, 2004b).

MODE OF ACTION

The mannan and mannoproteins represent 25-50% of the yeast cell wall and determine the cell surface properties (Lipke and Ovalle, 1998), which are believed to be the basis of the three primary modes of action of MOS observed in animal and poultry studies with Bio-Mos®: 1) adsorption (agglutination) of pathogenic bacteria containing Type 1 fimbriae; 2) modulation of the host immune response; and 3) enhancement of intestinal integrity (Spring et al., 2000; Shane, 2001).

Perhaps the best-studied and most well understood mode of action involves the competitive blocking of bacterial lectins. Adhesion of pathogens to the epithelial surface of the gut (colonization) is believed to be the first critical stage leading to infection. Mannose-specific lectins (Type 1 fimbriae) on the bacterial surface recognize glycoproteins (rich in mannose) on the host cell surface. The control of bacteria-mediated attachment has been proposed as a possible means of reducing enteric infection. Oyofo et al. (1989a) tested the effect of different sugars on the adherence of Salmonella typhimurium to epithelial cells from day-old chicks in vitro and found that mannose and methyl-α-D-mannoside were the most efficient in inhibiting adherence. They reported that mannose addition decreased the number of adherent bacterial cells to a defined intestinal surface by more than 90% when compared to a control with no carbohydrate added. In three follow-up in vivo studies, Oyofo and coworkers (1989b) observed a significant protective effect from supplementing mannose (2.5% w/v) in the drinking water of chicks for 10 days; salmonella-challenged control chickens were 78, 82 and 93% colonized whereas salmonella-challenged mannose-treated chickens were only 28, 21 and 43% colonized, in three respective trials. In other studies, addition of Bio-Mos® at significantly lower dietary inclusion levels (0.4% w/w) vs. the 2.5% w/v mannose concentration used by Oyofo et al. (1989a,b) resulted in the successful reduction of salmonella and E. coli in the ceca of young broiler chicks (Spring et al., 2000). This confirmed earlier in vitro studies that indicated differences exist in the ability of different mannose-based sugars to block pathogen attachment (Firon et al., 1983). Firon et al. (1983) demonstrated that compounds containing both α(1,3) and α(1,6) branched mannan (as found in the outer cell wall of S. cerevisiae) had approximately 37.5 times the binding capacity for E. coli as D-mannose. In another interesting study, Fernandez and coworkers (2000) demonstrated a reduction in colonization of S. enterica serovar enteritidis (PT4) in the ceca of young broiler chicks receiving the cecal contents from hens fed Bio-Mos® (2.5% w/w) through the diet. When chick diets were supplemented with the same Bio-Mos® level given to the hens, an even greater protection was observed, as demonstrated by fewer salmonella-positive birds observed, 11/24 (46%) compared with those fed mash alone (17/24 (79%). The ability of Bio-Mos® to interfere with the attachment of pathogenic bacteria in the gut raises the possibility that it could also inhibit the binding between bacteria that is required for plasmid transfer via conjugation (Newman, 2002). Lou (1995) demonstrated that dietary Bio-Mos® supplementation decreased the proportion of specific groups of Gram-negative antibiotic resistant fecal bacteria in swine. Work continues in this area to confirm these earlier findings.

Numerous studies have investigated the effect of Bio-Mos® on humoral and cell immunity. Whilst the exact mechanisms have not been completely elaborated, significant evidence has been accumulated to propose that Bio-Mos® plays a multi-purpose role in immune modulation. Dietary inclusion of Bio-Mos® has been shown to affect humoral immunity in turkeys by enhancing plasma IgG and bile IgA antibody levels (Savage et al., 1996). In another study, with sows receiving Bio-Mos® 14 days pre-farrowing and throughout lactation, higher concentrations of colostrum IgG and IgM were observed compared to the untreated sows (Newman and Newman, 2001). This increase in colostrum immunoglobulins was associated with the piglets from supplemented sows being significantly heavier at weaning. Non-specific cellular immunity has also been positively influenced in studies investigating macrophage activity. The stimulation of phagocytosis by Bio-Mos® has been demonstrated to be dose dependent in vitro (Sisak, 1995). This may be due to the presence of a mannose receptor (MR), which is involved in microbe recognition and phagocytosis in the absence of specific opsonization and acts like a true lectin in the lectin phagocytosis of micro-
organisms (Ofek et al., 1995). Mannose receptor is expressed on tissue macrophages, dendritic cells (mostly on Langerhans cells), endothelium, and rat microglia. In addition to acting as a scavenger of mannose-containing glycoconjugates on the surface of a wide spectrum of microorganisms such as *E. coli*, *Klebsiella pneumoniae* and salmonella, MR mediates their ingestion by macrophages (Mosser, 1994). MR is the main molecule involved in antigen recognition and the binding process in antigen-presenting cells (Engering et al., 1997). Therefore, activation of immune cells by yeast-associated mannan may facilitate antigen processing and serve to stimulate the initial stages of the immune response. Recently, there has been some evidence to suggest that MOS may suppress the pro-inflammatory immune response. Ferket (2002) induced an acute immune response in turkey poult's by intraperitoneal injection of LPS from *S. typhimurium* and measured fever response. Poult's fed a diet containing Bio-Mos® showed no fever response compared with the control (no additive) group, which experienced an increase of +0.4°C in body temperature. Greater control of the immune response, particularly the fever response, can be beneficial to the host in terms of energy savings, maintaining feed intake and reducing stress. Further studies are necessary to understand the highly complex and diverse effects the yeast cell wall mannans oligosaccharides have on the immune system of the host.

There is increasing evidence that MOS modifies the morphology and structure of the intestinal mucosa, although whether this is a direct or indirect (pathogen control) effect remains unclear. Early studies at Oregon State University demonstrated a reduction in crypt depth of turkey poult's fed diets containing 0.1 % Bio-Mos® through 8 weeks of age in three sections of the intestine comprising the distal half of the duodenal loop; Meckel's diverticulum and at the junction of the jejunum and cecum (Savage et al., 1997). These changes in crypt depth were correlated to a statistically significant increase in growth rate through eight weeks of age, suggesting an inverse correlation between the parameters measured. Santin and co-workers (2001) showed that inclusion of yeast cell wall at 0.2% in broiler diets aided in intestinal development with an increase in villus height during the first 7 days of life, and could be positively correlated with an improved body weight gain over the entire production period. Another detailed study evaluated the response of the intestinal mucosa of broiler chickens to Bio-Mos® included in sorghum/lupin-based diets at 0.0, 1.0, 3.0 or 5.0g/kg diet (Iji et al., 2001). Supplementation with the highest level of Bio-Mos® resulted in longer (P<0.01) jejunal villi. The RNA content of the ileal mucosal homogenate was significantly greater (P<0.05) in chicks receiving 3.0 and 5.0 g/kg Bio-Mos® diet than in other groups. The protein/RNA and RNA/DNA ratios in ileal homogenates were significantly (P<0.01) influenced by the presence of Bio-Mos® in the diet. This was not translated into increased mucosal growth or differences in digestive enzyme activities in the ileum. However, with Bio-Mos® inclusion, there were significantly greater specific activities of maltase (P<0.01), leucine aminopeptidase (P<0.05) and alkaline phosphatase (P<0.001) in the jejunum. Improvements in the intestinal mucosa with dietary supplementation of Bio-Mos® have been linked to a reduction in morbidity and mortality attributable to necrotic enteritis (Hofacre et al., 2003).

### Future uses for yeast cell wall

In recent years, glycobiology has become a rapidly developing sector of biotechnology, as researchers realize the potential for simple sugars and polysaccharides to affect biological mechanisms. The term ‘glycomics’ is the new buzzword of the biological sciences, building upon the more established areas of genomics and proteomics. Glycomics research divulges the biological implications of mono- and polysaccharide interaction with proteins and other cellular components to affect intercellular and cell-tissue interactions (Staples, 2003). The ubiquity and importance of carbohydrate interactions can be assessed by considering that 1% of human genes encode enzymes that contribute to glycosylation with several hundred glycosylation genes identified. The specificity and flexibility of glycosylation is not lost on drug manufacturers (Dove, 2001). Biotechnology companies have long used yeast to manufacture large quantities of proteins cheaply. However, yeast glycosylate proteins contain large quantities of mannose, allowing the immune system of the human or animal host to recognize the protein as foreign, thereby removing it from circulation and rendering the protein therapeutically useless. On the other hand, if the mannose in yeast glycoproteins could be replaced using ‘mammalian like’ sugar groups, such as sialic acid, there would be less chance of recognition by the host immune system and hence, an increased chance of successful delivery of the drug to its intended target.
From a biotechnological viewpoint, the presence of proteins at the outer surface of the yeast cell in direct contact with the environment is strategically attractive for drug manufacturers and the question of how proteins are targeted to this particular location has been intensively studied (Winzeler et al., 1999; Jung and Levin, 1999; Estrem and Skatrud, 2001). Improvements in yeast expression systems, coupled with the development of yeast surface display and refinements in two-hybrid methodology, are expanding the role of yeasts in the process of understanding and engineering eukaryotic proteins (Cereghino and Cregg, 1999). Display on the yeast cell is particularly suited to engineering mammalian cell-surface and secreted proteins that require endoplasmic reticulum-specific posttranslational processing for efficient folding and activity (Schreuder et al., 1996; Boder and Wittrup, 1997; Guo et al., 2002).

Some of the most exciting opportunities are emerging within research for fuel ethanol production. The production of ethanol directly from starch or cellulosic materials has remained the ‘holy grail’ of the fuel ethanol industry for many years (Dawson, 2003). To date, the limitation has been the inability of S. cerevisiae to ferment complex sugars to produce ethanol. Construction of novel S. cerevisiae yeast expressing glucoamylase, α-amylase, carboxymethyl-cellulose and β-glucosidase at the yeast cell surface has enormous potential for the industry to solve this problem (Ueda and Tanaka, 2000). In a series of experiments, Ueda and Tanaka (2000) demonstrated the ability of S. cerevisiae to efficiently ferment starch by introduction of genes encoding either a single heterologous enzyme or a multiple enzyme complex. The gene encoding glucoamylase, from Rhizopus oryzae, along with its secretion signal peptide was fused with the gene encoding the C-terminal region of β-agglutinin, from S. cerevisiae, and the construction was expressed in S. cerevisiae. Glucoamylase was demonstrated to be on the cell surface in its active form and anchored covalently to the cell wall. The engineered strain possessed the ability to utilize starch as the sole carbon source, as opposed to the inability of the parent strain. Further work improved the fermentation capability of this engineered strain through introduction of a truncated fragment of the β-amylase gene from Bacillus stearothermophilus. The engineered yeast co-displayed glucoamylase and β-amylase at the cell surface and had a more rapid growth rate on starch as the sole carbon source than the yeast strain only displaying glucoamylase.

In other research, simultaneous saccharification and fermentation of cellulose (β-glucan) to ethanol was demonstrated using an engineered yeast strain expressing endoglucanase II (from Trichoderma reesei) and β-glucosidase (Aspergillus aculeatus) on the cell surface (Fujita et al., 2002). The novel cellulose-degrading yeast strain could grow in a synthetic medium containing β-glucan as the sole carbon source and produced 16.5 g ethanol from 45 g β-glucan within 50 hrs. The yield in terms of grams of ethanol produced per gram of carbohydrate utilized was 0.48 g/g, which corresponds to 93.3% of theoretical yield.

Even though this technology is still in its infancy, the possibility to produce complex genetic constructions resulting in functional glycoproteins displayed at specific sites on the yeast cell wall is a reality. Clearly, the application of this technology in the area of feed, food and medicine will have a significant impact on our world in the near future. An example would include the ability to express specific feed degrading enzymes on yeast cell walls that could be targeted to specific substrates in the large intestine. The immobilization in the yeast cell wall would render the enzymes resistant to degradation through the upper digestive tract or through the rumen and allow for hydrolysis of a specific substrate at a specific site. The potential to maximize feed utilization, lower production costs and reduce environmental pollution in the animal production sector are of enormous economic benefit. In addition, the same yeast could be constructed with multipurpose adhesion properties; the addition of genes for glycoproteins that could target non-type 1 fimbriae possessing pathogens or target specific mycotoxins. The list of heterologous proteins expressed could be endless and the production of ‘designer’ cell wall materials in the near future is entirely feasible.

Conclusion

Carbohydrates have long been known to be an important dietary component, although traditionally have been seen as energy yielding molecules and structural components. Recently, studies have demonstrated that non-digestible carbohydrates play an important role in animal production and human health. Moreover, there is a growing recognition that non-digestible carbohydrates are more than an energy source for the colonic microflora but play a vital
role in cellular metabolism, protein structure and function, cell-to-cell communication and host immunity. The yeast cell wall is a natural source of two of the most potent oligosaccharides, β-glucan and mannan oligosaccharide. The functional properties of both components make them attractive for use in the human functional food sector. The data generated in the multitude of animal health and production studies indicate that there is merit in establishing clinical studies to investigate these functional properties. A greater understanding of cell wall composition and component functionality may lead to the development of new food, feed and pharmaceutical applications.

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Food animal agriculture: a few issues that will impact our future food supply

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Introduction: ‘food’ versus ‘feed’

To most of us human food and animal feed, although supplying nutrients, are two very different kinds of materials. This perception occasionally gets challenged when social scientists look at whether those folks with limited incomes are actually consuming pet foods. The authenticity of this claim is often challenged, but there does seem to be some truth to it. Certainly canned pet foods are commercially sterilized and meet the same regulations for low acid canned foods as regular human food. But the ingredients are obviously different and include ‘stuff’ that normally would not be considered human food. What many do not realize is that the rules governing what can be used as animal feed are at least as stringent as those for human food. The assumption that many people would probably make is that animals can eat almost anything they like unless specifically banned by the government such as the recent exclusion of certain animal products from the feed supply because of the incidence of BSE (mad cow disease). In fact, FDA can ban specific feed ingredients, but before anything can be fed to an animal, FDA must actively approve the ingredient. Thus, a whole infrastructure exists for regulating animal feed.

People working in animal agriculture often realize that in reality the rules governing feed are more stringent. This is not without a logical basis, as the feeding of animals is more complex because the growers have total responsibility for the health and safety of their animals. And, of course, the variety in an individual animal’s diet is much less than in human foods, where we probably have too many choices. Deficiency or excess of any nutrient in an animal feed becomes much more important and noticeable when that feed is the sole nutrient source. For humans, such problems are usually masked by the large variety of foods. Thus, getting the vitamin, mineral, antioxidant, calorie and other nutrients required in the correct amounts and proportions into animal diets is a challenge with feedstocks that in fact have significant variation and which when processed can become even more variable. Thus, feed analysis is often more critical than human food analysis and the flexibility of the nutritional labeling system for humans would simply not be tolerated by food animal producers. Would they permit a product that is labeled as 10 g/100 g protein to contain 8 g? What about 20 g in a legally labeled product? The rules for human food nutritional labeling permit those high variations to exist!

Of course laboratory and farm animal research has provided a great deal of our understanding of nutrition – both for the specific needs of those animals – and just as importantly for humans. Although our ability to extrapolate animal data to the human population is questionable and often controversial, the broad metabolic information that encompasses human nutrition has been defined. Our nutritional needs are not exactly like those of animals, particularly not of those animals that we normally use for research, but then again, we are not so far apart either, certainly in a nutritional sense. Thus, when interpreting nutritional science results, the normal scientific balancing act of not overstating or understating the meaning of the results is essential.

Food regulatory categories

When we examine the regulations for human food,
we must recognize that the government puts these foods and ingredients in many different categories. Substances that have a long tradition of being consumed as foods are not subject to regulatory oversight. The government does not purport to control the ‘food supply’. It focuses on the three main categories for traditional food ingredients: prior sanctioned compounds, food additives, and food components that are GRAS (generally recognized as safe).

Food additives go through the most stringent review. A great deal of evidence is required to establish both the safety and the need for a food ingredient. In the US this requires that a very complete petition be prepared for review by the FDA in order to obtain approval. The process can be slow and very expensive. The burden of proof of safety and efficacy is totally with the petitioner.

The GRAS approval process has changed in the past few years, but the onus remains on the petitioner to prove the safety of the ingredient. However, in the case of GRAS approval, the petitioner has more access to the consensus process that occurs within the scientific community. In recent years the FDA has encouraged more companies to ‘self-certify’ an ingredient as being GRAS. After the required literature review a petitioning company or organization can submit a GRAS petition to the FDA, who then have a short time in which to review the document. Unlike the Food Additive Petition process, if the FDA fails to respond in a timely manner, the current regulations give the GRAS petitioner de facto permission to proceed. One problem with all of the FDA procedures for foods is that a company, trade association or other petitioner must have a strong enough economic incentive to undertake the process. Thus a generic or more common material may not be worth enough to any one group to commit the financial resources needed to satisfy requirements for FDA clearance.

The prior sanctioned approach to new ingredients is basically a limited ‘grandfather’ clause that was created in 1958. It permits use in foods of materials for which there exists a letter from an appropriate federal agency (FDA or USDA FSIS) indicating that the material/process was approved by the government and can continue to be used. The only issue that occasionally must be clarified is how broadly is the ‘prior sanction’ defined? For example, nitrates/nitrites were clearly approved by the government for use in ‘meats’, but was poultry also considered meat for this application? Are all red meat products that use these ingredients covered, including those that were not conceived of until after 1958? There apparently were no letters approving use of nitrates/nitrites in poultry meat in 1958. De facto, both of the applications questioned above are covered by the prior sanction regulations, although the USDA has chosen to define the amounts that can be used in many meat and poultry products more narrowly. Is nitrite use in fish covered by prior sanction? Certainly fish have some very different chemistries than meat and poultry, so are not considered by FDA to be covered by the prior sanction status of red meat and poultry.

The other category of ingestible materials is drugs, both over-the-counter (OTC) and prescription drugs. The hurdles that companies producing these materials must pass are again quite rigorous, and much stricter than those for food additives.

As such, the US public was until recently pretty well protected by the government from consuming foods that were potentially dangerous. The FDA simply (in reality not so simple) was responsible for reviewing data submitted that clearly proved that the product was safe to place on the market.

DIETARY SUPPLEMENTS

However, there is one type of material whose status has always been a bit confused; and the laws regulating its use are not clearly covered by any of the above categories. This category is dietary supplements. The range of available dietary supplements is enormous, but the two issues of safety and efficacy have never been resolved legally for these products. In this wide range of products some are clearly above reproach, but others are marginal from safety or efficacy standpoints. Another issue is that of potency – is the amount promised on the package actually delivered in the product?

The government has relatively recently chosen to regulate these materials; and has created an infrastructure through the Dietary Supplement Health and Education Act of 1994 (DSHEA). This act establishes labeling standards, including the creation of a supplement nutritional labeling information panel that parallels the nutritional labeling panel for regular foods. The details are similar, but must be studied carefully in order to produce a ‘legal’ label. In addition, DSHEA and its subsequent regulations detail the nature of claims that can be made, and the full
implementation of regulations for label claims is still in process (see below). Probably more important with respect to consumer interest is the issue of who has the burden of proof of safety. For dietary supplements, unlike any other material in the food supply, the law places the burden of proving the material unsafe on the FDA. The recent banning of ephedra highlights the problems that appeared as a result. Companies can sell dietary supplements as long as the labeling, efficacy and marketing claim rules are obeyed – and if there are safety or health problems with these materials, then the FDA will have to take action. Given the large number of compounds that might need investigating, this is a serious challenge/hardship on the FDA; and given relatively limited resources, the FDA can realistically only take action on the most dangerous of materials, and this may be a very slow process. This is essentially what happened with ephedra. It is finally being withdrawn from the market, but long after people died or became ill.

Neutraceuticals and functional food ingredients are essentially dietary supplements added to the food supply in different ways. These can be considered as bioactive compounds with known (or supposed) biological or general physiological functions that go beyond simply providing essential nutrients. Many of these compounds come from other cultures where they are used in lieu of western medicine. Many have been used for years, possibly centuries. Those who study these compounds, either scientifically or pragmatically, often claim remarkable properties for them. Although it would be easy as scientists to ignore these compounds (as many scientists have and continue to do), others clearly feel strongly about their positive effects. As a result, many felt the need to prevent the government from prohibiting their use. This lack of faith in the traditional establishment, which includes the FDA, is clearly reflected in the DSHEA law. This development is actually most unfortunate, as it suggests that these compounds could not survive proper scientific testing. Many of these materials have real functionality, i.e., they really are neutraceuticals. But, on the other hand, a few are probably outright dangerous, and a few at best have no effect. The incentive to study them carefully is however lacking, although growth in consumer interest has certainly increased the scientific community’s involvement. With the government having the major responsibility for proving safety of these compounds, the nutraceutical industry has a disincentive to work with the academic community – who could at worst find something wrong with the product, publish it, and put FDA on the alert. Research, therefore, continues to lag at a time when scientific understanding of these materials could help both people and animals. Despite this, study in the food industry will continue as companies attempt to move the most promising compounds into the mainstream of foods. For many of these products, this trend is already apparent; and FDA has responded by further defining how such products can be marketed. In some cases the FDA has precluded marketing a product as a dietary supplement when the average consumer would consider it a direct analog to a regular food for which such ingredients are not normally a component. Thus a ‘drink’ could be a dietary supplement, but margarine with anti-cholesterol properties must be sold as a food.

The regulation of dietary claims will continue to develop as regulatory agencies try to strike the right balance. Currently working its way through the regulatory system is a plan to permit claims that do not meet the current high standard of significant scientific consensus. The FDA is trying to develop a system where claims based on less scientific consensus can be used, as long as the wording makes it clear what consensus such claims have. The issue currently is to determine the wording allowed for each type of claim.

Eventually the feed industry may be able to incorporate these functional materials into animal feed. Hopefully the additional process of getting materials approved for feed use once approved for human use will be fairly straightforward, although we need to recognize that the dose fed to animals might be higher and the potential for residues in edible tissues will always make the approval process for food animals more complicated.

Thus, the full benefit of using such materials remains to be developed. Thankfully companies like Alltech recognize the importance of this type of research for the potential benefit of the feed industry (and more recently for humans also) and have supported this type of research. Besides using the traditional peer-reviewed publication route, they have also taken a leadership role in making sure these findings are widely reported. These meetings sponsored by the company bring together people from many different industries within the scientific community to review and discuss work in these new areas.
Animal welfare

Another change occurring in animal agriculture that will influence the nature and future of functional foods centers on animal welfare issues. As the nature of the animal ‘experience’ changes in production agriculture, the benefits or detrimental impact of various functional ingredients will change. Improved animal welfare reduces stress by improving housing and environmental rearing conditions. Thus, those functional compounds that focus on remediation of excesses of current practice may not be necessary. On the other hand, the improved conditions may also allow benefits of certain functional ingredients to be more fully expressed. These compounds may then also be used to further improve the productivity of animal agriculture in a world that is currently trying to feed over 6 billion people and may need to feed over 9 billion before the century is out.

AUDIT PROGRAM

What has happened on the animal welfare front? As we learned two years ago at this meeting, from Bob Langert from McDonald’s, the three biggest fast food chains have begun paying attention to animal welfare issues. However, the supermarkets and other chain restaurants realized this was leading to establishment of a number of alternative standards. Since adopting a single standard would be more efficient, the Food Marketing Institute (FMI) (the trade association representing the supermarket industry) and the National Council of Chain Restaurants (NCCR) formed a scientific animal welfare committee to examine this important issue. The approach taken was to work with the main trade associations representing the major commodities (beef, dairy, pork, chicken, eggs, and turkeys), postponing others such as fish. In each case, the major trade association representing that industry sector was selected as the lead agency. Only where dairy is concerned, where the Milk and Dairy Beef Quality Center was selected, is there any question of the association being the leading trade association for that industry.

In many cases these trade associations already had or were working on animal welfare guidelines, which had resulted in guidelines established by a scientific or a scientific/industry team. The limitation with respect to this effort for most of the trade associations, however, was that these guidelines are voluntary and non-enforceable. In a few cases, programs were established that permitted producers to self-certify. In one case a program involving trained educators visiting the farm has been planned.

The FMI/NCCR committee has been using these guidelines as a starting point for their work. These documents have been critically reviewed by a group representing a different range of interests than the industry’s internal committees. In most cases, the FMI/NCCR accepted the industry standards, but in a few cases found adjustments were needed. These were first negotiated with the trade associations and in many cases the two groups came to agreement and the industry modified its standards. This negotiating process is ongoing.

The next step is to develop an audit program, which is outlined in Figure 1. First, the guidelines need to be translated into audit documents. This is not an easy task because the standards must be both quantitative and clear. They must also be very specific so that auditors can be trained to give nearly identical results. For this part of the program, the FMI/NCCR hired an outside auditing firm, which translated the approved guidelines into audit documents and is now training the auditors, supervising the audits, and collecting the data. They are preparing summaries of the audit data, maintaining the data base, and keeping the audit program operating. The first sets of auditors are being trained and the first audits are being scheduled.

A few important specifics need to be considered. First, the audit firm is training ‘outside’ auditors. The auditors are independent agents; and can be from other auditing firms or from government agencies. Auditors pay a training fee, take the course, and pass a subsequent written test; after which they start work with an already established auditor. Auditor prerequisites have been established. For on-farm audits the person must have an appropriate scientific college degree (e.g., biology, animal science, etc.), while for the slaughterhouse audits, the person must have an animal science degree.

Independent auditors bid for each ‘job’. A fee to cover the infrastructure is included. The facility being audited selects an auditor and makes arrangements for the auditor’s visit. The auditors will be instructed both by the FMI/NCCR program and by the individual facility with respect to biosecurity procedures.

Before undertaking a paid audit with the FMI/NCCR program, it is strongly suggested that producers...
use the documents and guidelines of their own trade association to do a thorough self-assessment. Once confident of in-house performance it is then time to bring in the formal auditing team.

In order to maintain consistency and skill level among auditors, it is expected that a reviewer hired by the audit firm will accompany the auditor on approximately one out of each ten audits to evaluate the work of the auditor. To further ensure the integrity of the process, the facility will have a right of appeal and a procedure for handling appeals is being established.

**Who owns the data obtained by the audit?**

The data is the property of the facility audited, regardless of who pays for the audit. The facility management/owner can then share the data as they
choose. Individual company data are only available to a few appropriate people in the audit firm. Only summary data will be made available to FMI/NCCR or the animal welfare scientific committee.

**How will retailers use the data?**

The data will be organized so that major and minor non-compliance will be clearly indicated. Retailers can then determine their own purchasing requirements based on their standards. There will NOT be a single ‘pass/fail’ standard. Each company must make its own decisions. The only audit ‘failures’ will be based on gross animal abuse, in which case the auditor is expected to terminate the audit and leave the facility immediately.

**Can non-compliance issues be resolved before the next full audit?**

Procedures will be put in place for correcting both major and minor non-compliance. For most minor non-compliance, the assumption has been made that the facility can submit appropriate paperwork to prove that corrections have been made. For major non-compliance the procedures will vary. Sometimes photos, paperwork and other forms of submitted material will be sufficient to establish that correction has been made, but in some cases the facility will need to be re-inspected.

How often a facility should be inspected is an issue still being addressed. Certainly slaughter facilities are likely to be inspected every year. Large production facilities are also likely to be inspected every year. On the other hand, cow-calf operations and other small, widely dispersed operations will require less frequent inspections and the procedures for these are still being discussed. It is possible that some sort of statistically based sampling regime may be necessary to keep the process cost-effective.

**Religious slaughter**

Specific standards covering both kosher and halal facilities have been written and are included in the program. Currently, these standards are based on the work of Dr. Grandin and support the material in the American Meat Institute guidelines, which have been established for over 12 years. However, it is clear that some kosher slaughter cannot meet these standards for religious reasons, and so a reconsideration of these needs will take place when these standards can be clarified and appropriate humane handling systems are in place.

**Alternative and organic agriculture**

Alternative and sustainable agriculture is becoming a more integral part of the US Agricultural and Land Grant system. With this heightened interest and the changing demographics in the US, many commodities are being raised and processed in new ways. For example there is renewed interest in sheep and goat rearing, which leads to new issues regarding slaughter in small facilities and on-farm slaughter for the ethnic and religious markets. As part of the Cornell Sheep and Goat Program and as part of a Northeast sheep initiative funded by USDA, we have been involved in efforts to improve the humane slaughter procedures for sheep and goats. This work has had three major thrusts. Firstly, to identify a knife appropriate for use in religious slaughter. Secondly, we have designed a slaughter pen for small-scale slaughter of sheep and goats that meets modern humane animal handling standards. Lastly, we have developed a poster that describes humane (halal) slaughter and makes suggestions on both animal handling and slaughter along with a reminder to deal with the offal in a socially acceptable way, suggesting composting as a method most in keeping with the need for an easy waste management system that is consistent with modern requirements. This poster is currently available in English and Persian and will be translated into Arabic, Urdu, and Spanish.

Although alternative agriculture does not necessarily imply organic cultural methods, certainly organic is an important subset. This grass-roots movement has gone in new directions in the past 10 years, and has come under the umbrella of government standards in the US and other countries. Basically the definition of organic, and what is and is not allowed as organic or in organic products, has been defined by the government, supposedly in conjunction with the ‘public’. However in many cases the additional paperwork burden and costs of participation in the government program will change the nature of who produces organic products. The facilities need to be large enough to justify the additional effort and costs. The rules are admittedly (the government's own admission) stricter than all previous rules, including those of the states that previously regulated organic labeling. In addition, the political nature of the process resulted in the US government precluding many of the potentially beneficial technologies that would permit organic agriculture to flourish. By eliminating genetically modified organisms and irradiation, the organic...
industry in the future will be limited in the crops they can grow and how well they can distribute organically-produced animal and vegetable products. Some current data have shown that in many cases a decrease in pesticide usage (the original emphasis of organic farming) and the use of more benign pesticides is possible with genetically modified crops. However organic producers are precluded from using GMOs. As part of the new regulations, we are seeing organic farming become big business and we are seeing big business take over organics – but is this what the core organic consumer really wants? Only time will tell how this will all settle out.
Mycotoxins in the food chain: a look at their impact on immunological responses

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Introduction

The immune system is an important defensive mechanism against invading parasitic organisms or foreign cells. The system is highly evolved in mammals and birds. In general, its complexity correlates with the evolutionary level of various animal species. In higher organisms, the system consists of specialized cells found throughout the body; these cells are localized in large quantities in certain organs such as the thymus, spleen, and lymph nodes. Cells of the immune system and cells of the hemopoietic system all originate in the bone marrow. The bone marrow stem cells later differentiate to perform specialized immunologic functions.

Immunotoxicology is a relatively new discipline, although the allergic responses to various chemicals have long been recognized. Chemicals, including mycotoxins, can either suppress or stimulate the immune system. Immunosuppression likely decreases resistance to a variety of infectious diseases and may even predispose the host to the expression and dispersion of cancerous cells. Stimulation of the immune system is not always desirable because it may lead to hypersensitivity (allergic) reactions. The mycotoxin-induced immunotoxicity has been reviewed earlier in detail (Bondy and Pestka, 2000; Sharma, 1985; 1991; 1993).

Complexities of the immune system

The immune system of mammals is highly complex, and various cells of this system interact to produce the desired effect. The immune system includes the innate immune functions that are inherent in different organs and do not require pre-exposure to antigens or chemicals. Some lymphocytes are natural killer cells that generally require no priming or proliferation to exert their effects. On the other hand, the generalized immune system usually confers acquired immunity after the organism has been in contact with infectious organisms or other antigenic determinants. Lymphocytes and macrophages are cellular units of both types of immune systems. The two major forms of lymphocytes, T cells and B cells, differentiate in the thymus and fetal liver, respectively. The T cells are involved in cell-mediated immune responses, such as delayed hypersensitivity reaction and immune surveillance against foreign or altered cells. Several subpopulations of T cells exist, e.g., cytotoxic T cells, helper T cells, and suppressor T cells. The B cells are primarily involved in the production of a variety of antibodies; however, these cells are often under the control of T cells. The T cells interact with one another or with other cells of the immune system via a variety of soluble factors called cytokines. In some instances direct cell-cell interactions are necessary to mount the optimal immune responses.

Macrophages are derived from monocytes and are present in various body cavities, such as pulmonary alveoli and peritoneum; they are also found in the lymph nodes and liver, as components of the innate immune system. Other peripheral leukocytes are often involved in various immunopathologic mechanisms. For acquired immunity macrophages are phagocytes that concentrate antigens and confer specific immunologic responses to various T or B cells and also remove cell debris.

The immune system interacts with other systems and is profoundly influenced by the central nervous system, both directly via innervations of lymphatic
organs and indirectly via neuroendocrine mechanisms. The cells of the immune system produce factors that influence the nervous system. Hormones, such as somatotrophin (growth hormone) and thymosin (thymic maturation factor), stimulate the immune responses, whereas steroids, including sex hormones, generally suppress the immune responses.

**Mycotoxins and public health**

Mycotoxins are metabolites produced by certain fungi that infest food crops and processed foods. The first recorded episode of mycotoxicosis in public health was recognized in the middle of the 19th century when ergotism was shown to be produced by ingestion of rye infected with *Claviceps purpurea*. In the first half of the 20th century, the ‘moldy bread disease’ or alimentary toxic aleukia (ALA) was associated with over-wintered wheat or grain in the former Soviet Union infected with various *Fusaria* species. The interest and intensive research in the problems with mycotoxins started with the incidence of Turkey-X disease in England, where the peanut meal used in poultry rations was found to be contaminated with aflatoxins produced by *Aspergillus flavus*.

Since the 1950s extensive research in the field of mycotoxins has provided information on a number of fungal products that are commonly associated with foods. The most common mycotoxins found in various foods are aflatoxins, fumonisins and trichothecenes, with occasional contamination with other toxins. Commonly encountered mycotoxins associated with various food commodities are listed in Table 1. Although the molds that produce these toxins are ubiquitous, the problem in foods can be easily managed by avoiding the fungal contamination and not consuming moldy or spoiled foods. Toxigenic fungi do not always produce mycotoxins. Their production is often aided by certain temperatures and humidity and stressful conditions to the fungi. Avoiding storage of foods at conditions favorable to mycotoxin production is highly valuable in controlling the toxic levels of these agents. Additionally, development of fungus-resistant crops has been somewhat useful in preventing the growth of toxigenic fungi.

In public health, the greater problem with mycotoxins is in developing countries rather than in the developed countries. In industrialized countries the food resources are plentiful, handling and preservation technology is well developed, and various regulations limiting the exposure to mycotoxins are in effect. On the other hand in developing countries, where food supplies are inadequate and storage facilities are not yet optimal, there is a greater chance for food spoilage and contamination by toxigenic molds. Even in developed countries; however, problems with mycotoxins frequently occur in livestock health.

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Commodity</th>
<th>Associated fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins</td>
<td>Peanuts, pistachios and other nuts, corn, cottonseed, cereals</td>
<td><em>Aspergillus flavus</em> A. <em>parasiticus</em></td>
</tr>
<tr>
<td>Fumonisins</td>
<td>Corn, other cereals</td>
<td><em>Fusarium verticillioides</em> F. <em>proliferatum</em></td>
</tr>
<tr>
<td>Ochratoxin</td>
<td>Legumes, cereals, coffee beans</td>
<td><em>Aspergillus ochraceus</em> Penicillium verrucosum</td>
</tr>
<tr>
<td>Patulin</td>
<td>Apples, grapes, other fruits</td>
<td><em>Penicillium expansum</em> <em>Aspergillus giganteus</em> other Penicillium and <em>Aspergillus</em> spp.</td>
</tr>
<tr>
<td>Trichothecenes</td>
<td>Wheat, corn</td>
<td><em>Fusarium tricinctum</em> F. <em>poeae</em> and other Fusaria and several other species</td>
</tr>
</tbody>
</table>

*Only common mycotoxins and representative fungi and commodities associated with them are indicated.*

**Prevalence of mycotoxins in foods**

Mycotoxins are inadvertent contaminants in foods as the fungi that produce them are fairly widespread. In spite of a large amount of research available for various mycotoxins, information regarding their occurrence in foods is limited. In many cases, the information is limited to periods when episodes of adverse human or animal health effects have been observed. Only a few of the mycotoxins are routinely analyzed in foods during surveys. The US Department of Agriculture and Food and Drug Administration have established limits for various mycotoxins in foods and feeds (Food and Drug administration, 2004). Still, food commodities sometimes exceed the allowed limits and may in fact be of concern in public health. Table 2 lists the levels of selected mycotoxins associated with different food commodities and their action levels established by US Food and Drug Administration. Additional information and primary sources of mycotoxin levels in foods are available from the recent reviews or other publications.
One of the public health concerns with the presence of mycotoxins in foods is that these toxins are relatively stable; once they are formed they can persist for a long time. Only a few of them are partially destroyed by cooking, pasteurization, or storage. The concentration of a mycotoxin may indeed increase during the fermentation process or storage if the toxigenic fungi are present in food. The mere presence of fungi however is not always indicative of the occurrence of mycotoxins, since the organisms produce these toxic metabolites only under certain conditions that are determined by temperature, humidity or a lack of normal nutrients for the mold. The occurrence of mycotoxins in agricultural commodities depends on factors such as geographical region, season, and the conditions under which a certain crop is grown, harvested and stored. There are no definite rules for if and when a mold will produce a mycotoxin. The fungal spoilage of crops and grains may be enhanced by drought, insect damage, cracked kernels during harvesting, and presence of excessive chaff in the harvested grain.

### Common health problems with mycotoxins in foods

#### AFLATOXINS

Metabolic products of *Aspergillus flavus* and *A. parasiticus* occur in foods in a number of susceptible commodities. Peanuts, corn, other nuts and grains are possible food commodities that may be contaminated. Aflatoxin B₁ is the most potent and prevalent of this group. Aflatoxin B₁ is activated *in vivo* by metabolizing enzymes to an epoxide, which is very reactive and binds to various biological molecules, including specific bases in the DNA. After dietary exposure aflatoxin B₁ is metabolized in the liver, the organ with a high level of metabolizing enzymes, and produces damage to this organ, including hepatocarcinogenesis. Indeed in some species, especially the rainbow trout, aflatoxin B₁ is the most potent carcinogen known. In the short term, aflatoxin B₁ causes necrosis in the liver and possibly damage to other organs such as kidney, heart, spleen and pancreas. In animals exposed to aflatoxin B₁ decreased productivity and high mortality due to infections are often the consequence.

Several episodes of aflatoxin B₁ poisoning in humans have been reported. These include both

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Commodity</th>
<th>Common levels (µg/kg)</th>
<th>Levels with toxic episodes (µg/kg)</th>
<th>FDA action levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins</td>
<td>Peanuts</td>
<td>2-6</td>
<td>30-125</td>
<td>20 ppb (µg/kg) in foods, 0.5 ppb aflatoxin M₁ in milk</td>
</tr>
<tr>
<td></td>
<td>Peanut butter</td>
<td>10</td>
<td>14-213</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Peanut candies</td>
<td>20</td>
<td>30-230</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Corn</td>
<td>&gt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fumonisins</td>
<td>Corn products</td>
<td>1-12</td>
<td>&gt;20,000</td>
<td>2 ppm in degermed dry milled corn</td>
</tr>
<tr>
<td></td>
<td>Corn (from various countries)</td>
<td>30-2,000</td>
<td></td>
<td>3 ppm in corn for popcorn</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 ppm in whole of partially degermed corn products, corn bran and masa</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>Corn, barley, wheat</td>
<td>&lt;3</td>
<td>&gt;25 (pork kidney)</td>
<td>No action level set</td>
</tr>
<tr>
<td></td>
<td></td>
<td>210-2,900 in bread flour (lumps)</td>
<td>3800 (barley in Czech Republic)</td>
<td></td>
</tr>
<tr>
<td>Patulin</td>
<td>Apple juice</td>
<td>9-146</td>
<td>1,000</td>
<td>50 ppb (µg/kg) in apple juice or food containing apple juice as an ingredient</td>
</tr>
<tr>
<td>Trichothecenes (DON)</td>
<td>Wheat flour</td>
<td>170-400</td>
<td>38,000</td>
<td>1 ppm for deoxynivalenol in finished wheat products</td>
</tr>
<tr>
<td></td>
<td>Cornmeal</td>
<td>100-400</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Popcorn</td>
<td>80</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bread</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Levels are only from some representative reports. Wide ranges in concentrations have been reported, particularly in contaminated samples.

general effects of this mycotoxin and carcinogenesis. Symptoms of suspected outbreaks of poisoning include jaundice, rapidly developing ascites, partial hypotension and death. In many parts of the world where hepatocellular carcinoma is prevalent, a high level of aflatoxin in the diet has been documented. However, many epidemiological surveys suggest the concurrent presence of hepatitis B virus and other mycotoxins such as fumonisins that may be contributory factors in addition to the aflatoxin B₁ (see later).

**FUMONISINS**

Fumonisins are toxins produced by *Fusarium verticillioides* and other *Fusarium* species commonly found on corn (Riley et al., 2001). Fumonisin B₁ is the most toxic and prevalent of these mycotoxins; its presence has been demonstrated in cornmeal, breakfast cereals and corn tortillas. Fumonisins have produced fatal diseases in livestock, including equine leukoencephalomalacia (ELEM, a rapidly developing brain degeneration) and porcine pulmonary edema (PPE or hydrothorax, lethal in a few days). An association of the presence of the fungus producing fumonisins and the presence of mycotoxins themselves has been reported with human esophageal cancer and primary liver cancer. The latter has been implicated when fumonisins are co-contaminants of foodstuffs with aflatoxins. Recently, carcinogenicity of fumonisin B₁ has been demonstrated in laboratory animals, including mice (hepatoma in females) and rats (renal carcinoma in males).

The species and gender specificity of fumonisin B₁ is puzzling and the basis for it has not been discerned. Fumonisin B₁ is a structural analog of one of the primary sphingolipid bases, sphinganine, and thereby competes for the incorporation of sphinganine into ceramide and ultimately in complex sphingolipids (Riley et al., 1998, 2001). A rise in cellular levels of sphinganine and sphingosine (dehydro sphinganine, another important free sphingoid base that is also a known signaling agent) is a uniform observation in all tissues exposed to this mycotoxin. These free sphingolipid bases (sphingosine and sphinganine) as well as their phosphate derivations are major signaling molecules that often possess paradoxical effects, characterized by either death or survival signals in cells.

The occurrence of fumonisins also depends on the fraction of corn produced during processing. For example, dry milling of corn kernels results in bran, flaking grits, grits, meal and flour. Fumonisins are found in greatest quantity in the bran fraction, followed in order by flour, meal, grits and flaking grits (lowest). This happens because fumonisins are concentrated in the hull and germ of corn kernel and milling fractions may contain different amounts of these parts. Degermed corn usually contains lower concentration of fumonisins than whole corn.

**OCHRATOXIN**

Ochratoxin A is a nephrotoxic fungal metabolite produced by certain species of *Aspergillus* and *Penicillium* that mainly contaminate cereals like corn, barley, wheat and oats. This mycotoxin, along with another nephrotoxic mycotoxin, citrinin, was implicated in Balkan endemic nephropathy that affected thousands of people in the middle of the 20th century in Eastern Europe. The disease was characterized by anemia, tubular proteinuria and hematuria. This toxin has been shown to be carcinogenic in rats and mice.

Toxicity to ochratoxin A has been reported in pigs after feeding grain with concentrations of this toxin as low as 0.2 ppm. Ochratoxin A is a potent inhibitor of protein synthesis and hence is immunosuppressant. Its teratogenic and mutagenic potential has also been shown in laboratory animals.

**PATULIN**

This mycotoxin is produced by *Penicillium*, *Aspergillus* and *Byssochlamys* molds that generally grow on apples. It can occur in significant amounts in apple juice or apple products. Although no major human or animal episodes have been recorded, patulins are toxic in animal feeding studies. The toxin is stable even after pasteurization, cooking or storage. Its presence has been reported in other food commodities, including bread, legumes, pecans, various fruits (including apricots, pears, grapes, etc.), fruit juices and cheese. It was once considered a potential antibiotic; however, it was abandoned due to lack of effectiveness and possible toxic consequences. It produces gastric irritation, nausea and vomiting upon ingestion. It is uniformly toxic in all mammalian species tested. The biochemical effects of patulin in cells include effects on mitochondrial respiration ultimately causing inhibition of electron transport systems.
Hazards to patulin exposure can easily be avoided by using only tree-picked fruits. Apples or other fruits that are damaged or rotten may not be used for making juice or other food products. The Food and Drug Administration established an action level of 50 µg/kg (50 parts per billion) in apple juice or any other foods that contain apple juice as one of the ingredients. This action level is based on single strength apple juice, one which is not concentrated, or the single strength apple juice component of the food, if the food contains apple juice as an ingredient.

**TRICHOTHECENES, INCLUDING DEOXYNIVALENOL (DON)**

One of the so-called trichothecenes, deoxynivalenol (DON, also commonly known as vomitoxin), is produced by molds of the genus *Fusarium.* *F. graminearum* is a common contaminant of grains including wheat, corn, barley and rye. Another major trichothecene, T-2 toxin, was implicated in the outbreak of alimentary toxic aleukia (ATA) that killed thousands of people in post-World War II Soviet Union. Trichothecene mycotoxins were also involved in the ‘red-mold disease’ intoxication in Japan. Contamination of grains with trichothecenes has been reported in the United States and Canada.

The symptoms of trichothecene toxicity include damage to skin or mucous membranes shortly after contact. Weakness, dizziness and incoordination may ensue shortly after the exposure. These symptoms are followed by bloody diarrhea, difficulty in breathing and bleeding from lungs or mucous membranes after exposure to aerosol containing trichothecenes. In livestock after a low level exposure, loss of appetite and decreased productivity are often reported.

Trichothecenes act by interfering with the ribosomes that are important in protein synthesis. Since protein synthesis is vital for many functions, including that of the immune system, the toxic manifestations of these mycotoxins are of importance in rapidly dividing cells and tissues. Damage to liver, spleen and other lymph nodes is often observed in cases of DON or other trichothecene poisonings.

**Mechanisms involved in immunotoxicity**

Little information exists on how various mycotoxins produce immunotoxicity. Some mycotoxins, such as aflatoxin B1 and fusarium T-2 toxin, inhibit protein synthesis and cell proliferation (Table 3). This inhibition may not be the primary mechanism involved

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Species</th>
<th>Toxic effects</th>
<th>Effects on immune system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins</td>
<td>Humans, all other mammals, birds, fish</td>
<td>Hepatotoxicity, bile duct hyperplasia, intestinal and renal hemorrhage, liver tumors</td>
<td>Reduced lymphoproliferation, delayed hypersensitivity, phagocytosis, antibody formation against T-dependent antigens, increased infections</td>
</tr>
<tr>
<td>Fumonisins</td>
<td>Human, pig, horse, mouse, rat,</td>
<td>Pulmonary edema in swine, leukoencephalomalacia in horses, liver and kidney damage, esophageal and hepatic cancer in humans (?)</td>
<td>Decreased lymphocyte blastogenesis; decreased antibody titers after antigens or vaccines. Decreased splenic and thymic cellularity and interleukin-2 production (in female mice)</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>Human, swine, dog, duckling, chicken, rat</td>
<td>Nephrotoxicity, enteritis, liver damage, teratogenesis, renal carcinogenesis</td>
<td>Decreased phagocytosis, cellular depletion of lymphoid organs, transient immunostimulation</td>
</tr>
<tr>
<td>Patulin</td>
<td>Birds, mammals (cat, rabbit, cattle)</td>
<td>Lung hemorrhage, capillary damage, convulsions, brain edema, carcinogenesis (mammals and birds)</td>
<td>Reduced DNA synthesis in lymphocytes, decreased peripheral leukocytes, altered distribution of lymphocyte subpopulations</td>
</tr>
<tr>
<td>Trichothecenes</td>
<td>Human, pig, cattle, chicken, horse, rat, mouse, dog</td>
<td>Vomiting, diarrhea, bleeding, dyspnea, itching, rash, blisters, leukopenia</td>
<td>Increased infectivity, decreased lymphoproliferation and antibody formation, decreased macrophage cytokine production</td>
</tr>
</tbody>
</table>

*Only selected symptoms and effects on immunological responses have been indicated. Many mycotoxins are also likely to stimulate production of specific antibodies.*
Mycotoxins and immune response

in their immunotoxic effects; both have selective effects on various subpopulations of lymphocytes. Several mycotoxins are cytotoxic to lymphocytes in vitro, perhaps because of their effects on membranes (including those involving lymphocytic receptors) or interference with macromolecular synthesis and function. Cytochalasins (mycotoxins isolated from moldy rice) are highly cytotoxic and act on cytokinesis (perhaps by binding to the filamentous actin), but their immunotoxic potential has not been ascertained.

Mycotoxins can indirectly influence the immunologic functions. Some of the compounds are neurotoxic or cause other organ pathology, and these compounds may activate the endocrine mechanisms. The stress-induced release of corticosteroids inhibits immune functions. Fusarium T-2 toxin, which acts via such mechanisms, is discussed later.

Some mycotoxins or their metabolites may be highly reactive in mammals and may bind to or destroy tissues. The immune system can also respond to altered proteins or to other biological molecules formed by binding with reactive chemicals, although no experimental evidence exists of this mechanism involving mycotoxins. Antibodies against mycotoxins conjugated with proteins have been produced and are utilized for analyses for mycotoxins using immunoassays.

The influence of exogenous chemicals on immune responses may be highly variable and a mycotoxin may increase, decrease, or fail to affect the response, depending on the testing protocol and dose.

Impact of mycotoxins on immune responses

AFLATOXINS

Immunomodulation by aflatoxin B₁ has been investigated in detail. A comprehensive description may be found elsewhere (Bondy and Pestka, 2000; Sharma, 1991). In most species, resistance to infection is reduced by simultaneous exposure to aflatoxin B₁. Effects of aflatoxin B₁ are primarily on the cell-mediated immune functions; however, T cell-dependent humoral responses are also adversely affected (Reddy et al., 1987; Reddy and Sharma, 1989). Generally the humoral responses that are T cell-independent are not affected by low doses of aflatoxins. The immunosuppressive effects of aflatoxin B₁ can be explained on the basis of DNA binding of the resulting epoxide derivative of aflatoxin B₁, thereby interfering with cell proliferation and protein synthesis.

In humans, the investigations dealing with aflatoxin B₁ and impaired immune function are limited. In some parts of the world the exposure to this mycotoxin can be considerably high. Exposure to aflatoxin B₁ was reported to be high in Thailand, Swaziland, and Mozambique (45, 43, and 222 ng/kg/day, respectively). In Mozambique the mortality due to hepatoma in hepatoma B virus-antigen carriers was twice that observed in other countries. The actual risk of similar carriers is the same throughout the world. It was proposed that since Mozambique had a high average intake of aflatoxin, the resulting loss of immunoprotection may account for higher viral-induced hepatoma incidence (Lutwick, 1979). A similar increase in primary hepatic carcinoma was suggested in certain provinces of China when people were exposed to high levels of aflatoxin B₁ and fumonisin B₁ (Ueno et al. 1997); the involvement of immune system in this carcinogenesis is uncertain as both of these mycotoxins are complete carcinogens.

There have been limited studies on the effect of aflatoxin B₁ on cytokine production. The expression of macrophage-derived cytokines, namely interleukin (IL)-1α, IL-6 and tumor necrosis factor α (TNFα), was suppressed in mitogen-stimulated macrophages derived from aflatoxin B₁ exposed mice (Dugyala and Sharma, 1996). This report suggested that the effect of aflatoxin B₁ was greater on macrophages than on other types of immune cells. Macrophages as scavengers may engulf large amounts of aflatoxin-bound macromolecules and thereby are selectively sensitive to the toxin.

FUMONISINS

There have been a number of investigations involving fumonisin B₁ and immune responses (reviewed by Bondy and Pestka, 2000). Fumonisin B₁ generally inhibited lymphocyte blastogenesis in cells obtained from exposed mammals; poultry was relatively resistant. Inconsistent effects of fumonisin on immune functions have been reported. Decreased antibody formation to injected antigens has been reported in turkeys, pigs, calves or even rodents; however, the levels of fumonisins used in these studies have been fairly large.

Much of the studies in laboratory animals and cell lines have been inconclusive with regards to
fumonisin-induced immunomodulation. The results depend on the protocol of testing and sequence of exposure to toxin and antigens. In a recent report it was observed that fumonisin B₁ altered immune functions in female BALB/c mice, but the males were totally refractory (Johnson and Sharma, 2001). It has been established that fumonisin B₁ causes accumulation of free sphingoid bases, sphinganine and sphingosine, by interfering with their conversion to ceramide (Riley et al., 2001) and somehow interrupts the cell cycle (Johnson et al., 2003). The free sphingoid bases and their phosphates are important signaling agents in cells, the bases and their phosphates usually having opposite outcomes for cell survival. Cellular signaling is critical in mounting immune responses; indeed fumonisin B₁ has been used as a tool to define the role of ceramide in signaling in immunocompetent cells (lymphocytes). Sphingoid signaling in various physiological processes is becoming relevant and should be further investigated. The possible involvement of immunological responses in the pathological outcome after fumonisin B₁ treatment is discussed later in this report.

OCHRATOXINS

Ochratoxin A, a nephrotoxicant widely encountered as a food contaminant, has been investigated for its immunologic effects. The biochemical mechanism of ochratoxin poisoning involves interference with macromolecular synthesis, increased lipid peroxidation and diminished mitochondrial respiration. This mycotoxin is fairly cytotoxic and causes atrophy of gastrointestinal lymph nodes after oral ingestion (reviewed by Bondy and Pestka, 2000). Systemic investigations suggest that exposure to ochratoxin A results in the inhibition of cellular, humoral or innate immune responses; however, many of these effects are produced at exposure levels that are also nephrotoxic. The immunotoxic effects have been demonstrated in poultry, pigs, rats and mice. In some studies immunostimulation, perhaps as a consequence of tissue damage, was also reported.

PATULIN

Patulin has been investigated with regard to the immune system only to a limited extent (Llewellyn et al., 1998). It is less toxic than other mycotoxins; however, effects such as altered number of splenic T lymphocytes, diminished serum immunoglobulin concentrations, decreased delayed hypersensitivity responses and increased neutrophil numbers were reported. The observed changes in cellular phenotype distribution of immune cells may or may not indicate ultimate effects on immune functions. It is suggested that a limited human exposure to foods contaminated with patulin may be of little consequence for the immunologic functions.

TRICHOTHECENE MYCOTOXINS

The prevalent trichothecenes, T-2 toxin, deoxynivalenol (DON), oxynivalenol, and diacetoxyscirpenol, have been evaluated for their immunologic effects to a great extent (reviewed by Sharma and Kim, 1991). This group of mycotoxins was important for immunological investigations as T-2 toxin was implicated in the onset of alimentary toxic aleukia in the Russian population. Leukopenia is a consistent observation after trichothecene exposures. T-2 toxin is perhaps the only mycotoxin with known immunotoxic dysfunction in humans.

Exposure to T-2 toxin and deoxynivalanol results in severe depletion of T cells. In routine immunotoxicological testing in rodents, both increase and decrease of immunologic functions have been reported depending on the protocol employed. T-2 toxin was immunosuppressive in murine in vivo and in vitro models (Taylor et al., 1985; 1987). Although it is presumed that trichothecene mycotoxins are immunotoxic, the mechanism of their action is not well understood. These mycotoxins are inhibitors of protein synthesis; however, the high susceptibility of T cell function cannot be totally explained on this basis. The T-independent responses are generally less sensitive to T-2 toxin effects. Oral exposure to T-2 toxin in mice results in inflammatory lesions in the forestomach (Taylor et al., 1989), leading to systemic endotoxemia. These effects were associated with increased hypothalamic catecholamine levels and increased peripheral corticosterone concentrations, thereby implicating a possible role of hypothalamic-pituitary-adrenal axis in the resulting immunosuppressive responses.

Deoxynivalenol and T-2 toxin have been investigated for their effects on cytokine production by immunocompetent cells. However, effects were not consistent. T-2 toxin caused increased expression and production of IL-2, IL-3 and interferon γ in splenocytes from T-2 treated mice, but a decrease in
IL-1α, TNFα and IL-6 was noticed (Dugyala and Sharma, 1997). Deoxynivalenol, however, caused an increase in TNFα production by macrophages (Sugita-Konishi and Pestka, 2001; Yang and Pestka, 2002).

The deoxynivalenol-induced nephropathy in mice can be demonstrated by passive injection of deoxynivalenol-induced IgA monoclonal antibodies (Yan et al., 1998). The findings imply that increased systemic IgA after deoxynivalenol may be implicated in pathogenesis of this mycotoxin in kidney, suggesting the involvement of an immune mechanism in the ultimate pathologic outcome to this mycotoxin.

Immune mechanisms in fumonisins B₁-induced murine hepatotoxicity

We have recently investigated the role of immune pathways in the induction of hepatotoxicity in mice after treatment of animals with fumonisin B₁. Systemic treatment of mice with fumonisin B₁ caused consistent induction of the pro-inflammatory cytokines, TNFα and interferon γ, in liver (Bhandari and Sharma, 2002; Bhandari et al., 2002b). Indeed the exposure to fumonisin B₁ caused localized activation of the cytokine network, implying that innate immune responses are important in the hepatotoxic outcome (Bhandari et al., 2002). The toxicity of fumonisin B₁ in murine liver was decreased in mice lacking either tumor necrosis factor receptor (TNFR)-1 or TNFR-2 (Sharma et al., 2000; 2001). The hepatotoxicity was also ameliorated in mice lacking interferon γ expression (Sharma et al., 2003). Recently we have also observed that fumonisin B₁ hepatotoxicity in mice is totally abolished in NZB/NZW-F1 mice that are spontaneously prone to systemic lupus erythematosus (unpublished observations). The accumulation of free sphingolipid bases was identical in both lupus-prone mice and wild-type controls. The lupus-prone mice are deficient in the production of inflammatory cytokines, including TNFα and interferon γ, thereby further supporting the hypothesis that production of cytokines by innate immune cells in liver is responsible for the ultimate pathological outcome of hepatotoxicity in susceptible animals. Similarly, when mice were injected with anti-thymine antibody (anti-Thy 1.2) together with fumonisin, no hepatotoxicity was observed (unpublished observations).

Miscellaneous mycotoxins in food

There are a number of other mycotoxins that can occur in certain circumstances as food contaminants. Many of these are toxic and also influence the immunologic functions. These include citrinin (a nephrotoxic mycotoxin), rubratoxins (that cause liver and kidney toxicity and diffuse hemorrhage), macrocyclic trichothecenes (a large family of mycotoxins with varied toxicity), secalonic acid (an antineoplastic but teratogenic toxin), cytochalasins (mycotoxins that bind to actin and also inhibit hexose transport in cells), ergotoxins (potent vasoconstrictors), penicillic acid, etc. Few or conflicting reports of these miscellaneous mycotoxins with respect to their effects on immune functions are found in literature. Because of low probability of their occurrence in foods at present, these are not discussed here.

References


Antioxidant activity of hydrolyzed whey, soy, and yeast proteins

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Introduction

Proteins as functional ingredients are widely used in the food and animal feed industries to improve product physical attributes and to enhance nutritional value. Proteins from a variety of sources, such as soy, whey, yeast, and animal by-products, have also been subjected to enzymatic hydrolysis to further enhance their general functional performance or to impart specific functionalities in prepared foods (Adler-Nissen, 1986). Studies have shown improved functional properties, including emulsification, gelatinization, foaming, and water-binding capacity of proteins after they are partially hydrolyzed either by acids or by enzymes (Kinsella, 1976; Feeney and Whitaker, 1977). The improvement has been attributed to increased exposure of reactive amino acid side chains as well as to the production of reactive short peptides.

While the benefit of hydrolyzed proteins (i.e., peptides or peptide mixtures) as texture-modifying agents in food is well established, it was not fully recognized until recently that certain hydrolyzed proteins or peptide fractions also have biological functions, notably antioxidant activity. The antioxidative effect of protein hydrolysates was initially observed by Doan and Miller (1940), who showed that trypsin-treated milk had improved oxidative stability. Bishov et al. (1967) subsequently presented the first report on antioxidant activity in an acid hydrolysate of soybean proteins. Later studies by Lee et al. (1980), Chen et al. (1995), and more recently, Pena and Xiong (2001; 2002; 2003), also revealed antioxidant activity of soy and whey protein hydrolysates prepared by enzymatic digestion. In addition to soy and milk proteins, hydrolysates of other proteins have been found to exert inhibitory effects on lipid oxidation and to extend the shelf life of food products. Among these reported antioxidant proteins are hydrolyzed egg white albumin (Tsuge et al., 1991), casein (Lim and Shipe, 1972; Rival et al., 2001), capelin meat (Amarowicz and Shahidi, 1997), elastin (Hattori et al., 1998), gelatin (Kim et al., 2001), and myofibrillar protein (Saiga et al., 2003).

The antioxidant capability of hydrolyzed proteins, in addition to their other physicochemical functionalities, offers potential for expanded application in the food industry. In light of the increasing consumer demand for ‘natural’ food ingredients and at the same time, for high product quality and palatability, protein hydrolysates offer an attractive alternative to other natural antioxidants that have the exclusive role of inhibiting oxidative processes.

Preparation and characterization of protein hydrolysates

Protein hydrolysates can be manufactured by either acid treatment or enzyme hydrolysis. The latter has the advantage of producing specific peptides or a mixture of different peptides with a particular functionality. Enzymatic hydrolysis is also more controllable than acid hydrolysis and does not destruct labile amino acids (Adler-Nissen, 1993). In developing antioxidative peptides from whey (WPI) and soy (SPI) protein isolates, we employed three commercial crude proteases – Protease ‘A’ (endoproteinase from Bacillus licheniformis), Protease ‘P’ (Bacillus protease complex), and Protease ‘F’ (endoprotease and exopeptidase from Aspergillus
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oryzae), and four pure enzymes – pepsin, papain, trypsin, and chymotrypsin (Pena and Xiong, 2001, 2002). By varying hydrolytic conditions (pH, temperature, time, and substrate heat pretreatment), a range of peptides varying in molecular weight, hydrophobicity and solubility were produced.

Of the three crude enzymes used, Protease A was found to be the most effective in degrading whey proteins as determined by SDS-PAGE (Figure 1). β-Lactoglobulin, the predominant whey protein component, was almost completely hydrolyzed after 30 min regardless of preheating. Within the same time period, α-lactoalbumin was also mostly degraded, while the higher molecular weight proteins (IgG, serum albumin, etc.) vanished entirely. Hydrolytic products included numerous small peptides (mostly <6 kDa). Native whey protein components were somewhat resistant to hydrolysis by Proteases P and F; however, with heat treatment, all whey proteins became highly susceptible substrates. For hydrolysis with pure enzymes, native β-lactoglobulin was resistant to pepsin but became more susceptible after heat treatment. All the protein components in preheated whey samples were highly susceptible to papain, trypsin and chymotrypsin, with most degraded into fragments with molecular weights less than 1 kDa (Pena and Xiong, 2001).

![Figure 1. SDS-polyacrylamide gel electrophoresis of whey protein isolates treated with Proteases A, P and F. Molecular weight (MW) standards shown are in kDa. β-Lg: β-lactoglobulin; α-La: α-lactalbumin. (Adapted from Pena and Xiong, 2001).](image-url)
Size-exclusion chromatography was performed to separate hydrolyzed proteins into peptide fractions based on their molecular weights. These fractions were divided, based largely on their chromatographic profiles, into three for control (nonhydrolyzed), four for Protease A, and five for both Proteases P and F, with the sizes of the fractions ranging from 1.5 kDa to 66 kDa. Amino acid analysis showed a varying distribution of the 20 common amino acids in the different peptide fractions.

As to soy proteins, both conglycinin (7S) and glycinin (11S) and their subunits were susceptible to the three crude enzymes. Hydrolysis with Protease A yielded peptides that fell into two molecular mass groups: 60-90 kDa and less than 15 kDa (Pena and Xiong, 2002). The degree of hydrolysis increased slightly with incubation time. On the other hand, hydrolysis with Proteases P and F produced a range of peptides that were widely distributed (15-100 kDa).

The concentration of free amino groups in hydrolyzed soy protein ranged from 1.3 µmol/mg for Protease P treatment to 5.1 µmol/mg for Protease F. Native, unhydrolyzed soy protein contained 0.7 µmol free amines per mg of protein.

Yeast protein extracts were prepared by autolysis of components of a yeast strain obtained from Alltech Inc. During the autolytic process, endogenous enzymes break down yeast cell proteins into peptides and amino acids, and nucleic acids into nucleotides (Romero and Gomez-Basuri, 2003). These derivatives are normally used as flavor enhancers. Electrophoretic analysis of the yeast extract with a 10% polyacrylamide gel detected no distinct protein bands at >10 kDa, but the biuret measurement showed 26.4% reactive peptides, suggesting that the hydrolytic product consisted of savory short peptides (<2 kDa), some of which may be di-, tri- and oligopeptides, along with free amino acids. The amino acid composition of the yeast hydrolysates is shown in Table 1.

### Antioxidant activity of protein hydrolysates and peptides

**WHEY PROTEINS**

Nonhydrolyzed WPI was slightly inhibitory of lipid oxidation, according to the TBARS assay (thiobarbituric acid-reactive substances) in a liposomal oxidizing system (Pena and Xiong, 2001). Upon hydrolysis, the WPI samples exhibited various antioxidant activities (suppression of TBARS formation), depending on the enzymes involved and whether or not the substrate (WPI) was preheated. Hydrolysis with Protease A and the four pure enzymes (pepsin, papain, trypsin, chymotrypsin) did not significantly change the antioxidant activity. However, hydrolysis of WPI by Protease P produced pronounced antioxidant activity, which increased with hydrolysis time up to 6 hrs where a 32% inhibition (P<0.05) was observed (Figure 2). For Protease F treatment, a 39% reduction (P<0.05) in TBARS was found for heated WPI that was hydrolyzed for 1 hr. Both the antioxidant protein hydrolysates were formulated (2%) into cooked pork patties before storage, and the analysis of conjugated dienes (hydroperoxides) showed a significant inhibition (up to 44%) of hydroperoxides during storage (Pena and Xiong, 2003). Except for the Protease P treatment, no relationship (P>0.05) can be established between degree of hydrolysis and antioxidant activity. Furthermore, although the heat regimen slightly increased degree of hydrolysis, it did not consistently impact antioxidant activity of the protein hydrolysates.

For comparison, the antioxidant properties of six commercial WPI hydrolysates were also analyzed. Although the procedures and conditions for preparing the hydrolysates (e.g., proteases used) were not known, all the samples (8-26% degree of hydrolysis) showed antioxidant activity. This was demonstrated by 5-24%...
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inhibition of TBARS, compared to the nonhydrolyzed control or the control that contained no whey proteins (Pena and Xiong, 2001).

The results suggest that antioxidant activity of WPI hydrolysates was probably inherent to the characteristic amino acid sequences of the peptides, which were a function of the enzyme specificity (Browdy and Harris, 1997). Indeed, the antioxidant activity assay of the peptide fractions, prepared by size exclusion chromatography from the protein hydrolysates that exhibited strong antioxidant capability, provided compelling evidence that the antioxidant power was not equally distributed in different peptide fractions. The percent inhibition of TBARS in the liposomal oxidizing system by peptides varied from merely 8% to more than 50%.

To further elucidate the nature of the antioxidant effect by the protein hydrolysates, TBARS inhibition (%) was correlated with the amino acid composition and average peptide size from each of the antioxidative peptide fractions. The results showed that TBARS inhibition was positively correlated (P<0.05 or 0.01) with the serine, histidine, arginine, threonine, alanine, valine, lysine, isoleucine, and leucine content, but was overall inversely related to the peptide size (Table 2).

The data were in general agreement with previous findings that certain amino acids, including most of those shown in Table 2, can be antioxidative (Marcuse, 1960; Karel et al., 1966). Vulnerability of these amino acids (or residues on polypeptides) to free radical attack seems to stem from the low energy involved in the process. The reaction, in which amino acids are being oxidized in place of unsaturated lipids, would in effect stabilize or neutralize the radicals and consequently, inhibit propagation of lipid oxidation. Østdal et al. (1999) showed that protein or peptide radicals tended to be remarkably stable and can live for a long time, e.g., >30 minutes.

Table 2. Correlation coefficient (r) between TBARS inhibition and amino acid concentration and peptide size of hydrolyzed whey protein.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Correlation coefficienta</th>
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<tbody>
<tr>
<td>Lysine</td>
<td>0.894**</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.537*</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.583*</td>
</tr>
<tr>
<td>Aspartic acid/asparagine</td>
<td>0.159</td>
</tr>
<tr>
<td>Glutamic acid/glutamine</td>
<td>0.305</td>
</tr>
<tr>
<td>Glycine</td>
<td>-0.004</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.830**</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.778**</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.825**</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.406</td>
</tr>
<tr>
<td>Proline</td>
<td>0.314</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.709*</td>
</tr>
<tr>
<td>Serine</td>
<td>0.666*</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.325</td>
</tr>
<tr>
<td>Valine</td>
<td>0.916</td>
</tr>
<tr>
<td>Peptide size</td>
<td>-0.772**</td>
</tr>
</tbody>
</table>

a Significance level: *P<0.05, **P<0.001
Despite their antioxidant activity, none of the WPI hydrolysates was as effective as the propyl gallate treatment (0.01%) that inhibited TBARS formation by 85%. The results were not surprising because protein hydrolysates could contain both antioxidative and pro-oxidative peptides and amino acids prepared under certain specific hydrolytic conditions (Karel et al., 1966; Adler-Nissen, 1993; Chen et al., 1996). Presumably, the antioxidant effects of some peptides and amino acids were offset by other peptides and amino acids that acted as pro-oxidants.

SOY PROTEINS

All the SPI hydrolysates exhibited antioxidant activities, as indicated by the TBARS assay in a liposomal oxidative model system (Pena and Xiong, 2002). The hydrolysates prepared from heated SPI with chymotrypsin and Protease F were particularly effective in inhibiting lipid oxidation (Figure 2), e.g., suppressing TBARS by 59-64% by samples that were hydrolyzed for 1 hr. When formulated into cooked pork patties, both protein hydrolysates (2%) inhibited hydroperoxide production by 50% (Pena and Xiong, 2003). Comparison of hydrolyzed WPI and SPI samples indicated an overall stronger antioxidant activity for the SPI hydrolysates.

It was of interest to note that intact SPI (no enzyme treatment), whether or not heated, already possessed substantial antioxidant power (reducing TBARS by 36%). Wu and Brewer (1994) also reported that unhydrolyzed soy protein was capable of inhibiting lipid oxidation and retarding rancidity odor development in ground beef during storage. It has been shown that most soy protein isolates contained residual antioxidative phenolic compounds, typically in the 1-1.5 mg/g range, including isoflavonoids that are capable of quenching free radicals (Seo and Morr, 1984). These presumable phenolics ostensibly contributed to the observed antioxidant activity of native SPI. For nonheated SPI, hydrolysis with Protease F elicited a less antioxidative effect when compared with heated SPI. In fact, hydrolysis of native SPI by chymotrypsin weakened its antioxidant potential. A similar result was obtained with papain hydrolysis. The type of proteases chosen and the conditions of protein hydrolysis (substrate, time) are ostensibly some of the most critical factors determining the antioxidant activity of soy protein hydrolysates.

YEAST PROTEIN EXTRACTS

The antioxidant activity of two Alltech yeast extracts was evaluated using both the FRAP assay (ferric reducing/antioxidant power), as described by Benzie and Strain (1996), and the TBARS measurement (Sinnhuber and Yu, 1977). The FRAP analysis was done with dilute yeast extract solutions, while the TBARS analysis was conducted on cooked beef patties. The FRAP value of yeast extract 2 was higher (P<0.05) than that of yeast extract 1 at every concentration level (Figure 3). Since yeast extracts 1 and 2 have an identical amino acid composition (Table 1), the difference in FRAP values may be attributed

![Figure 3. FRAP values (obtained at 4-min assay) of yeast extracts at different concentrations.](image-url)
to the peptide composition and perhaps also the nucleotides that probably differed between the two yeast extracts. Both yeast extracts were able to inhibit lipid oxidation in cooked beef patties during refrigerated storage, and the inhibition increased (P<0.05) with the level of extract addition (Figure 4). At the end of storage (7 days), TBARS values in samples with 1.5% yeast extract 1 and yeast extract 2 were 20.2% and 17.3%, respectively, less than the control. Except for the level of 0.1%, all concentration levels within each yeast extract group were able to suppress TBARS production after 3 days (P<0.05). The storage study revealed no significant difference between the two yeast extract samples.

The antioxidant power of the yeast extracts is presumably related to the presence of antioxidative amino acids that may be free or in a bound form. For example, histidine, a widely reported antioxidative amino acid (Taylor and Richards, 1980; Chen et al., 1996; Wade and Tucker, 2001), accounted for 0.95% (w/w) of the yeast extract weight or 2.01% of total amino acid weight (Table 1). It may have a role in the overall antioxidant activity of the yeast extract samples. Furthermore, the sulfur-containing amino acids cysteine and methionine, comprising 1.52% and 1.74%, respectively, of total amino acid weight, were expected to contribute to the reducing power of the yeast protein samples. It is worth noting that the contents of essential amino acids lysine (8.13%), leucine (6.75%), isoleucine (4.74%), and valine (6.20%), which were responsible for much of the antioxidative activity of whey protein hydrolysates (Table 2), were also high in the yeast extracts. The overall antioxidant power of both yeast extracts may be a combined effect of the multiple active components present in them, i.e., the individual amino acids, the profile of the peptides, and possibly also nonprotein substances such as nucleotides.

**Possible antioxidant mechanism**

The ability of protein hydrolysates to inhibit deleterious changes caused by lipid oxidation appears to be related to certain amino acid residues and the specific amino acid sequence of hydrolytic peptides. Amino acids such as methionine, histidine, lysine, tryptophan, and proline have previously been shown to act as antioxidative agents although in certain particular cases they may be pro-oxidative (Marcuse, 1960; Karel et al., 1966; Jung et al., 1995; Chen et al., 1996). Correlation analysis in the present study indicated a positive role played also by leucine, isoleucine, valine and threonine in inhibiting lipid oxidation. The study by Taylor and Richardson (1980) with a linoleate emulsion-hemoglobin oxidizing system concluded that among the common amino acids, cysteine was the one that clearly displayed an antioxidative efficacy. The antioxidative effects of small peptides in oil or metal-catalyzed liposomal suspensions have been demonstrated (Yamashoji and Kajimoto, 1980). In particular, carnosine and anserine, histidine-containing antioxidative dipeptides, were capable of sequestering metal ions and scavenging...
free radicals (Chan et al., 1994; Lee et al., 1998; Decker et al., 1992, 2000; Wu et al., 2003). It appears that while some free amino acids may be antioxidative or pro-oxidative, the efficacies will change when the amino acids become constituents of the oligo- or polypeptide chains. For native whey, soy and yeast proteins, the folded molecular structure may be less conducive to interaction with radicals and other pro-oxidative or oxidizing agents. However, hydrolysis, which exposes reactive amino acid residues or peptide patches, would enable the products to readily react with oxidizing agents, forming more stable radical or nonradical species (Østdal et al., 1999), thereby inhibiting oxidation of lipids and other susceptible constituents in raw and processed foods.

Conclusions

Enzyme-hydrolyzed whey and soy proteins and autolyzed yeast extracts contain antioxidant components as demonstrated in liposome and cooked meat patty model systems. The specific antioxidative efficacy of the hydrolyzed proteins varies with the peptide composition and differs among hydrolysates. Because the size, composition, and physicochemical nature of peptides can be controlled by varying the time of hydrolysis, by heat treatment of the protein substrates, and by proper selection of hydrolytic enzymes, it may be possible to develop effective food grade antioxidants from common food proteins. The presence of antioxidant activity in prepared protein hydrolysates would allow them to be marketed and utilized as premium food ingredients because not only can they improve the physicochemical attributes of food products (texture, water-binding, flavor enhancement, etc.) but they also can increase product stability and shelf life.

References


Equine topics
A novel, knowledge-based concept for performance diagnosis and training adjustment in horses

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Introduction and rationale

When comparing the scientific approaches and efforts that are currently applied to improve performance capability in all fields of human athletic competition, it is more than obvious that scientific approaches to improve performance, training conditions or analyse training status of horses are dramatically ignored, even by top athletes in the equestrian sport. We are missing knowledge-based concepts to monitor equine fitness and strategies to improve performance capabilities based on a defined and testable training program. According to statements of top athletes, not even the performance of our top international competitors is analysed by scientific methods during an important sport event. This is partly due to the fact that most scientific research on horses focuses on medical aspects. Nevertheless, it is hard to understand why in this day and age the equestrian sport relies on traditional methods, with coach and rider relying alone on ‘gut feelings’ and experience.

A couple of years ago, the Equestrian Research and Study research group at the German Sports University of Cologne decided to transfer the methods known from traditional human sport disciplines to equestrian sports. The focus was first on the human part, the rider. But of course success in equestrian sport events does not exclusively depend on the capability of the rider. It is the performance of the ‘companion’ horse that is in most cases essential for success. In this respect, the focus of our research has changed. We have started to develop new discipline-specific methods of performance diagnosis and training programs for the equestrian sport, with the horse as the centre of interest. This novel concept and the analytical methods are the main focus of this paper. The general idea is to create concepts for all disciplines in equestrian sports, since factors such as endurance, speed, power and coordination of movements are doubtless of general interest. In the beginning it was hard to find the acceptance and thus enough horses to generate statistically sound data. Meanwhile we had the chance to start our scientific research in cooperation with one of the top athletes of the German International Jumping Rider team, so the data presented have been in most cases obtained with horses that perform in jumping events.

Exercise program to improve endurance

Our first approach was to create a training program to improve endurance in horses, as endurance is the basis for all disciplines. We took advantage of experience from human sports. In human sports a so-called step program is the method of choice, where the athlete is challenged in step 1 by a time-limited exercise (usually a run) under controlled conditions (i.e. a given speed) and has a defined resting period (walking). The athlete would then go through more cycles (steps 2 – x) under conditions of increasing intensity (i.e. higher speed), while the resting time is kept constant. Usually this two-step program is repeated four times. The performance of the athlete is monitored by measuring heart rate and lactic acid production as described below, and the program is designed in a way that matches individual abilities. The design of this program, especially the challenge step, is usually the very sensitive part. If the athlete is not sufficiently challenged, the program is useless, however overtaxing is devastating, but will be revealed by physiological measurements such that the program can be adapted.
Our step program for horses is as follows: Before the test begins, the resting heart rate and the resting lactic acid concentration are determined. Horses are allowed to warm up before the first step is started. Step 1 includes a gallop with a speed of 325 meters/min for 5 min. A short rest (1-2 min) follows and the first aliquot of capillary blood is taken. During Step 2, the speed is increased by 50 meters/min (i.e. to 375 meters/minute), but the challenge time is kept at 5 min. Two more steps are performed, each time increasing the speed by 50 meters/min and keeping the challenge time constant. It should be emphasized at this point, that the speed of the first step and the number of steps strongly depends on the endurance capability of the respective horse, and must be adjusted individually.

ESTABLISHING PHYSIOLOGICAL MEASUREMENTS THAT DESCRIBE ENDURANCE

Performance during an athletic competition certainly depends on a discipline-specific endurance. The endurance factor is important in dressage and jumping and gains further importance in cross-country or long distance racing events. Despite the differing demands of these events, general performance parameters can be established to monitor endurance in each discipline. With a glance at what has been learned from human athletes performing in gymnastics, heart rate and lactic acid accumulation in the blood should be the parameters of choice. It can be considered as a general rule in sports physiology that during exercise the heart rate increases. If the athlete is well-trained, this heart rate drops when he returns to moderate movement or rest. The rate of decrease in heart rate has been shown to reflect a good training condition in humans.

Thus our first approach was to establish a means of continuously recording heart rate of horses during exercise. As in humans, electrodes were fixed on the body of the horse and linked to a recorder enabling us to monitor this physiological parameter during exercise. Figure 1 shows the simple experimental set-up and a recording that was done during a regular horseback riding lesson. Heart rate reached top values of 120-130 beats/min during the lesson and when walking the heart rate came to steady-state levels within 5 min. When challenging horses in the step program, heart rate values of up to 180 beats/minutes and more were observed (Figure 2).

Maximal heart rates are certainly individual characteristics. Even in horses we observed heart rates up to 200 beats/min and more during Grand Prix Jumping events, so they cannot be considered the only marker of fitness.

Lactic acid accumulation is another marker that is generally applied to assay exercise and endurance in humans (Hollmann and Hettinger 1990). The biochemical explanation is that during exercise, needed energy is provided by two metabolic principles, which will be considered in brief.

The general energy source, glucose, is metabolised to pyruvate by a pathway called glycolysis. This 11-step pathway creates two molecules of ATP, the energy currency of the body (which can be spent for energy-consuming work such as muscle contraction), and one reduction equivalent. It is important to note

![Figure 1](image_url)
that these reactions will occur without the presence of oxygen, thus they are called the anaerobic reactions of glucose metabolism. Pyruvate is then metabolised by the citric acid cycle to carbon dioxide, generating reduction equivalents that can be used in the respiration chain, which uses these reduction equivalents to reduce oxygen to water, thus generating much more energy in the form of ATP than glycolysis. This is the aerobic part of glucose metabolism. During exhaustive exercise when the rate of oxygen consumption due to the work of the respiratory chain gets high, oxygen cannot be delivered by haemoglobin in the appropriate amount, so the ATP-energy-producing respiratory chain is not able to provide enough ATP for the muscle cell. Thus, the cell relies on its oxygen-independent pathway, glycolysis (Figure 3). Glycolysis can only proceed if the reduction equivalent produced in its reaction steps is converted to oxidation equivalent; and only if the accumulating pyruvate, which cannot enter the citric acid cycle, is removed. Thus the cell reacts by converting pyruvate to lactic acid with the help of the reduction equivalent. The amount of lactic acid transferred to the blood can be considered as a marker of exhaustive work.

**Figure 2.** Time course of the heart rate of a horse challenged by our step test.

**Figure 3.** The time process of aerobic and anaerobic reactions (Clayton, 1991).
A study by Hodgson and Rose (1994) revealed that horses, independent of athletic discipline, usually perform under aerobic conditions. In humans it is known that well-trained athletes accumulate lower concentrations of lactic acid in the blood than non-trained individuals (Hollman and Hettinger, 1990). Lactic acid is usually assayed in a small aliquot of capillary blood in anticoagulant solution, which is kept on ice until laboratory analysis (Figure 4).

During the step test blood samples were taken to monitor lactic acid accumulation (Figure 5). All horses accumulated lactic acid to some extent, depending on fitness level. From human studies it is known that the blood serum lactic acid concentration influences performance capabilities (Heck and Rosskopf, 1994). Humans get ‘sour’ when they reach a well known threshold of lactic acid accumulation and can no longer perform with coordinated movements. In humans the threshold is 4 mM lactic acid; and we observed the same threshold for horses as well. These results point to the fact that during exhaustive exercise horses react with lactic acid formation, the extent of which depends on the individual performance capacity of the horse; and this parameter must be considered during controlled exercise in order not to overtax the horse. On the other hand, in the timed schedules applied, heart rate and lactic acid accumulation will reflect the endurance capability and the progress of the individual horse.

While the assay methods described above can be generally applied, the step test can certainly be modified. Recently we have started to develop step tests for jumping horses, which include a standardized course. In addition, we monitored some of our jumping horses during international events by measuring heart rate and lactic acid formation to better estimate the stress during these big events.

**Outlook**

**THE SPEED, POWER AND COORDINATION FACTORS**

In many equestrian sports speed, power and coordination are additional factors that determine success. Certainly these factors are interrelated. We have recently started to precisely monitor the speed and acceleration capacity of jumping horses with a LA VEG-speed analyser, which is based on laser technology. The question is whether a special program to improve acceleration that is known from other athletic programs (i.e. short sprints followed by a resting period) is effective in horses as well. In preparation are programs to improve and actually monitor power with the help of weight-adjustable sledges. But this special program must be discipline-specific, because horses performing in dressage,

Figure 4. (A) An aliquot of capillary blood is taken from the horse and transferred to (B) Eppendorf tubes filled with anticoagulant solution and kept on ice.
jumping or cross-country all have individual demands. One consideration is: does the horse need maximal power throughout the performance, or do we need to improve ‘maximal power on the point’? In other words, does a horse that performs in dressage for 5 min need maximal power during all this time, or just when performing power-consuming manoeuvres like passage?

The most complex factor is coordination. To analyze the various kinetic aspects during jumping (i.e. jumping distance to the fence, time of last contact with the soil or technique while jumping) high-speed video movies were recorded and evaluated (Figure 6). The scientific question is whether training can improve these kinetic parameters.

Taken together, we have collected a broad spectrum of analytical methods to analyse and monitor horses during sport events. Training programs known to have successfully improved performance in the human sports were adapted. All these approaches definitely must be accompanied by training diaries, regular veterinary check-ups, weight controls and specialized feeding programs.

Figure 5. Time course of lactic acid formation in horses challenged with the same step test as in Figure 2
(Bojer and Schulz, 2000).

Figure 6. 2-D kinetic analysis of horse and rider (Pozzo et al., 2001).
A novel, knowledge-based concept for performance diagnosis and training

References


Physiology and feed formulation: the proper role of carbohydrates in the equine diet

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Introduction

The statement, “I want a low carbohydrate feed for my horse”, has become popular in recent years. The reasons, or potential reasons, for wanting a low carbohydrate horse feed could be many. One possibility is a simple carry over from the current human dietary trend of reducing carbohydrate intake. The Atkins Diet is one popular means of reducing carbohydrate intake in human meals. With millions of dollars spent on media advertisements for restaurants with ‘Atkins Friendly’ menus, each of us is at least reasonably familiar with the low carbohydrate concept stressed in this diet. It follows then that with an estimated 64\% of Americans overweight and at any given time 29\% of men and 44\% of women trying to lose weight, one could conceive that popular food culture may be influencing the horse owner’s thought processes when buying horse feed. However, there may be more legitimate reasons for a horse owner to seek a low carbohydrate feed. Included in the list of reasons are the desires to influence or modify behavior, or sensitivity to so-called carbohydrate diseases including tying-up, Cushing’s Disease, laminitis, insulin resistance (IR), obesity and osteochondrosis dissecans (OCD). But before we recommend that our clients reduce or eliminate carbohydrate from the equine diet, we should understand more about carbohydrates and their proper role in horse diets.

Classification of carbohydrates

The equine digestive tract is anatomically classified as that of a non-ruminant herbivore (Frape, 1986). This digestive arrangement allows feedstuffs to be broken down with enzyme digestion in the small intestine and microbial fermentation in the cecum and colon, commonly referred to as the hindgut. Plant material (hay/pasture), cereal grain and commercial grain concentrate, the cornerstones of modern equine diets, consist of a wide array of carbohydrates and may contain up to 75\% carbohydrate (Pagan, 1998). However, not all of this carbohydrate is digested or absorbed in the same manner within the equine digestive tract.

On a simplistic basis, carbohydrates found in the equine diet can be crudely divided into two types, structural and non-structural. Structural carbohydrates are typically found in the cell wall of the plant and are often referred to as fiber. The major carbohydrates associated with the cell wall are cellulose, hemicellulose and lignin. These carbohydrates are represented on a laboratory analysis report as neutral detergent fiber (NDF). Baled hay, mature pasture grass, beet pulp and soybean seed coats are good sources of structural (fibrous) carbohydrate (NRC, 1989). Structural carbohydrates are resistant to enzyme digestion in the small intestine and must be fermented by bacteria in the horse’s hindgut (Frape, 1986). Bacterial fermentation of fiber yields volatile fatty acids (VFAs). VFAs are absorbed from the hindgut and are transported to the liver where they are converted to energy substrates for the horse. The overall digestibility of fibrous carbohydrate is quite variable, depending on the distribution of cellulose, hemicellulose and lignin in the carbohydrate fraction. Since lignin is non-digestible by bacterial fermentation (Frape, 1986) the higher the degree of lignin present the lower the overall digestibility. Thus, as plants mature and increase in lignin content,
digestibility is decreased. The overall digestibility of NDF in good quality forages by horses varies from 40-50% (Pagan, 1998).

Non-structural carbohydrate (NSC) is carbohydrate associated with the inner portion of the plant cell, or plant cell contents. The plant cell includes NSC along with protein, lipids, organic acids and soluble ash. NSC is made up of sugars, disaccharides, starches and fructans. In warm season grasses (C4 plants), starch is the primary storage carbohydrate, whereas in cool season grasses (C3 plants) fructan is the primary storage carbohydrate. As a practical point, commonly fed legumes such as clover and alfalfa do not contain fructan, and store carbohydrate as starch. Enzymes in the horse’s small intestine break down sugars and starch to monosaccharides (simple sugars) that are absorbed and circulate in the blood as glucose. Fructans are resistant to mammalian enzyme digestion and must be fermented by bacteria in the horse’s hindgut (Suzuki and Chatterton, 1993). Sugar and starch are highly digestible, greater than 95%, within the length of equine digestive tract. Bacteria located in the hindgut ferment any starch or sugar that is not digested by enzymes in the small intestine. Unfortunately, fermentation of sugar, starch and fructan by hindgut microorganisms can produce lactic acid, and the resulting acidosis can destroy the environment within the hindgut leading to death of the microorganisms and health concerns such as colic and laminitis (Richards et al., 2003).

The extent to which NSC is digested in the small intestine is dependent on the source of the NSC, processing, level and rate of intake, time and frequency of forage feeding and individual horse digestive characteristics (Meyer et al., 1993). Briefly, starch originating from oat grain is more digestible within the small intestine compared to starch from corn or barley. This difference is related to the microscopic structure of the starch granule, and thus the surface area available for enzyme digestion. Processing (rolling, crimping, grinding, etc.) generally increases the digestion of starch in the small intestine, again increasing the surface area for enzyme digestion. As level of starch intake increases, the small intestine digestibility decreases due to rate of passage of feed material. The feeding of forage following a meal rich in sugar and starch will increase rate of passage and decrease small intestine digestibility of sugar and starch. Finally, differences in the ability of individual horses to either digest or absorb sugars from the small intestine has been reported (Richards et al., 2003).

The NSC content of feeds can be determined with modern laboratory methods (e.g. Dairy One, Forage Analysis Laboratory, Ithaca, New York, USA). Feeds with large amounts of NSC include commercial grain concentrates containing large amounts of oats, corn and barley and any product containing high amounts of molasses. Forages such as pasture and baled hay also contain NSC, but the amount is quite variable, ranging from 1 to 40% of dry matter. The NSC content of forages is influenced by type of plant, environmental temperature, light intensity, drought stress, plant fertility, and rate of drying during the curing process (Smith, 1973). Briefly, C3 (cool season) grasses are generally higher in NSC than C4 (warm season) grasses. Cold stress, especially in C3 grasses, increases the NSC content. Photosynthetic capacity, and hence production of sugar, is directly correlated to light intensity and duration. NSC concentration will be lowest in early morning if the night was warm enough to allow the sugars produced the previous day to be utilized by respiration. Further, shading or cloud cover of grass plants reduces NSC content. Drought stress is a stimulus for NSC accumulation in grass. Drought limits plant respiration causing the accumulation of NSC. It is widely accepted by forage researchers that nitrogen and phosphorus deficiency causes an increase in concentration of NSC in both grass and legumes. Finally, the faster plants dry after being cut for hay production, the higher the NSC content. Plants will continue to respire and burn off sugars until plant moisture content is below approximately 40%. The only method to determine the NSC content of a feed ingredient is laboratory analysis. Visual characteristics and type of hay are not predictable indicators of NSC content.

**Carbohydrate-related diseases**

In the last ten years, many equine disease treatment protocols have investigated the role of dietary carbohydrates. Several disease states may be precipitated or exacerbated when high levels of carbohydrate are being fed. The following is a brief description of several disease conditions and the role that carbohydrates may play in the treatment or prevention of disease.

Tying-up is a condition associated with the equine musculature system. Tying-up is also known by the following names: Azoturia, Monday Morning Disease, and Exertional Rhabdomyolysis. It is expressed
forage is scarce. During periods in which forage is adaptation for time when conditions are harsh and at times when forage is plentiful provides a survival development of additional body fat (relative obesity) variable availability of forage (grass). The temporary metabolism for survival based on the seasonally University of Missouri, evolution equipped the equine horses. According to Dr. Philip Johnson from the induce laminitis in research animals.

Grain overload is commonly used as a method to with overeating of grain (starch) the best known cause. Laminitis is associated with a number of risk factors, painful for the animal and often debilitating. The complications of this disease are laminitis and diabetes mellitus, with both complications made worse with high starch diets (Geor, 2001). Laminitis is a disease condition associated with the hoof and its attachment to the bony structure of the equine foot. In a bout of laminitis, inflammation of the laminae alters tissue blood flow to the sensitive living tissues that attach the coffin bone to the hoof wall (Redden, 2001). This causes separation of the non-sensitive hoof wall and the laminae such that the coffin bone becomes detached from the hoof wall and sinks toward the ground. This disease is extremely painful for the animal and often debilitating. Laminitis is associated with a number of risk factors, with overeating of grain (starch) the best known cause. Grain overload is commonly used as a method to induce laminitis in research animals.

Obesity is a very common problem for modern horses. According to Dr. Philip Johnson from the University of Missouri, evolution equipped the equine metabolism for survival based on the seasonally variable availability of forage (grass). The temporary development of additional body fat (relative obesity) at times when forage is plentiful provides a survival adaptation for time when conditions are harsh and forage is scarce. During periods in which forage is relatively unavailable, the body fat stores, which were never intended to become excessive, are depleted in order to provide energy for survival. Under many modern horse management systems, the combination of feeding starch-rich rations over many years and protracted periods of stall confinement or lack of adequate exercise tend to lead to the acquisition and maintenance of substantial body fat in the domesticated horse. The development of obesity in both humans and horses directly causes insulin insensitivity (Johnson, 2002). Insulin insensitivity is known to lead to endothelial cell dysfunction, which is involved in the pathogenesis of vascular complications in humans. Insulin insensitivity is currently being studied as a cause of laminitis in horses, and future feeding recommendations may require these horses to eat low carbohydrate (low glycemic) diets.

Osteochondrosis is one of a number of growth anomalies that affect the skeleton of young horses. Osteochondrosis can be very serious in that it often results in debilitating lameness that in many instances reduces or eliminates athletic performance (Jackson, 2003). The cause of osteochondrosis is multifactorial. The proposed causes of osteochondrosis include genetic predisposition, rapid growth rate, mechanical stress and trauma, nutrition excess, mineral imbalances and endocrine factors (McIlwraith, 1996). New research is re-focusing on hyperglycemia and/or hyperinsulinemia as a cause of osteochondrosis (Glade et al., 1984; Ralston, 1995; Pagan 2001). Specifically, scientists are looking to determine if certain foals that experience an exaggerated and sustained increase in blood glucose and insulin in response to a carbohydrate meal may be predisposed to development of osteochondrosis (Pagan, 2001). Hyperinsulinemia has been reported to be a potential endocrine factor that contributes to equine osteochondrosis (Henson et al., 1997). If this does prove to be a predictable cause of osteochondrosis, then feeding foals carbohydrate-rich diets may be contraindicated.

Minimizing carbohydrate intake

From the available information on several diseases thought to be sensitive to carbohydrate in the diet, the desire to create a low carbohydrate diet is really a need to control the amount of NSC (sugar, starch and fructan) the horse consumes. Structural carbohydrate (fiber) should not be a target for elimination since fiber is essential for proper function and motility of the horse’s digestive system.
Practicing equine nutritionists commonly recommend that horses receive a minimum of 1.5% of body weight per day in dry forage to provide essential fiber. However, some work is now being done to correlate a more predictable fiber value, neutral detergent fiber (NDF), to voluntary dry matter intake of horses (St. Lawrence et al., 2001).

If it is necessary to reduce the NSC intake in an equine diet, the following steps will help accomplish that goal. The first step in minimizing the amount of NSC (sugar and starch) in a horse’s diet is to determine the NSC content of the feed. Unfortunately, this is not information that can be found on the feed tag. A quality lab for determination of NSC is the Dairy One Forage Lab, Ithaca, New York. The cost of analysis is approximately $15 for a combination of sugar, starch and fructan analyses and approximately $26 for a more complete nutrient analysis including minerals and full carbohydrate fractions. Table 1 lists the average NSC content of feed ingredients in the US as published in the Dairy One Ingredient Library. The reader should note that NSC content of the ingredients can be quite variable due to growing conditions previously outlined. With a range of up to 35% of dry matter, NSC content of forages certainly requires that we not rely on ‘book’ values for average NSC content.

<table>
<thead>
<tr>
<th>Feed</th>
<th>NSC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stored forages</td>
<td></td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>11.4</td>
</tr>
<tr>
<td>Mixed hay, mostly legume</td>
<td>12.2</td>
</tr>
<tr>
<td>Mixed hay, mostly grass</td>
<td>13.5</td>
</tr>
<tr>
<td>Grass hay</td>
<td>13.3</td>
</tr>
<tr>
<td>Oat hay</td>
<td>23.0</td>
</tr>
<tr>
<td>Straw</td>
<td>12.0</td>
</tr>
<tr>
<td>Fiber products</td>
<td></td>
</tr>
<tr>
<td>Almond hulls</td>
<td>45.5</td>
</tr>
<tr>
<td>Beet pulp</td>
<td>12.2</td>
</tr>
<tr>
<td>Citrus pulp</td>
<td>29.0</td>
</tr>
<tr>
<td>Soybean hulls</td>
<td>7.2</td>
</tr>
<tr>
<td>Cottonseed hulls</td>
<td>5.2</td>
</tr>
<tr>
<td>Cereal grains and molasses</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>73.2</td>
</tr>
<tr>
<td>Barley</td>
<td>63.1</td>
</tr>
<tr>
<td>Oats</td>
<td>50.7</td>
</tr>
<tr>
<td>Molasses</td>
<td>58.4</td>
</tr>
</tbody>
</table>

The second step in minimizing the amount of NSC in the diet is to only feed the amount of grain or supplement necessary to maintain body condition and ensure proper dietary fortification of protein, vitamins and minerals. Since grains and grain concentrates with molasses contain high levels of NSC, minimizing the amounts of these products included in the diet will minimize total NSC intake. Unfortunately, many horse owners are quick to eliminate all grain products from the diet and therefore also eliminate the source of essential vitamins and minerals that are associated with the grain concentrate portion of the diet. If grain is to be eliminated from the diet, a low-intake protein, vitamin and mineral supplement should be added to provide these essential nutrients. If horses do require large amounts of grain, it should be recommended that clients feed grain concentrates with moderate to low levels of NSC. The use of dietary fat, either in the feed or top-dressed onto the feed, has been shown to slow the rate at which the stomach empties and to control the surge of glucose into the blood following a meal (Pagan et al., 1995). Therefore, feeding high fat feeds or adding fat to the diet may help to eliminate many carbohydrate problems. However, long-term feeding of fat may lead to impaired glucose tolerance in ponies (Schmidt et al., 2001), and is contraindicated in obese horses.

The final step in reducing NSC intake is to consider the forage portion of the diet. Overgrazed, stressed pasture grass can contain an abundance of NSC and should be avoided with horses known to have laminitis, episodes of tying-up, insulin resistance, Cushing’s Disease or obesity. Pasture grass should be replaced with baled hay or other fibrous carbohydrate sources such as hay cubes or hay pellets, appropriately tested for NSC content. Since visual characteristics and type of hay are not reliable indicators of carbohydrate content, new research is focusing on methods to reduce the carbohydrate load of hay after it has already been baled. Watts (2003) has reported on one method to reduce the water-soluble carbohydrate (sugar) content of hay. In that research, Watts soaked fifteen samples of various species of hay in water. She reported the average amounts of sugar reduction after 30 and 60 minutes of soaking in cold water were 18.9% and 30.7%, respectively. The average amount of sugar removed in 30 minutes of soaking in hot water was 29%. This research offers the first step in methods to practically limit the sugar content of baled hay.

**Summary**

In summary, there are health concerns that dictate the need to feed low carbohydrate diets to certain
Since there is an abundance of different carbohydrates in horse feed, the choice of which carbohydrates to limit is critical. Carbohydrates found in equine feeds can be roughly divided into two types, structural and non-structural. Structural carbohydrates are often referred to as fibrous carbohydrates and should not be eliminated from the diet. Non-structural carbohydrates (NSC) are commonly referred to as sugars, starch and fructans and can be associated with several so-called carbohydrate diseases. NSC is the carbohydrate that can be minimized in the equine diet. Methods to minimize NSC content of the diet include analysis of feed ingredients to determine NSC content, reduction in intake of ingredients with high NSC content, and modification of forage intake including elimination of stressed pasture and the soaking in water of baled hay to rinse water-soluble carbohydrate away.

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Relevance of the NRC to today’s horse industry

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Current status of the horse industry

The horse industry entered the new millennium during a period of strength and popularity not seen since possibly the beginning of the previous century. This upward trend followed several years of a declining horse population beginning in the mid-1980s. The general decline of the horse industry following a serious economic downturn was reflected in high horse slaughter during this time, with over 300,000 horses per year being processed by abattoirs. Since then, horse slaughter has declined by 75-80% and has so far shown no sign of increasing despite the current economic slowdown and the general consensus that horse populations are increasing in most parts of the United States. The improvement in the status of the horse industry is indicated by strong feed sales by several major horse feed manufacturers and the continued strength of sales of equine related goods and services such as trucks, trailers, tack, pharmaceuticals and veterinary and farrier services. Although the public auction sale prices of the highest quality select horses have declined significantly since 2001, a reflection of world events and the depressed stock market, the entry level, companion horse, youth and amateur horse markets remain quite strong. Fewer low-quality horses exist today compared to two decades ago, and today’s horse owner, both new and experienced, values quality, convenience and the aesthetic properties of feeding and caring for horses like never before. The Farm Bureau’s recent emphasis on the equine industry through appointment of county, state and national equine committees suggests a fundamental change in the perception of the equine industry by traditional agricultural organizations. As the swine, dairy, poultry and beef industries consolidate and fewer livestock producers maintain membership in professional agricultural organizations, the horse industry membership will continue to be cultivated by these organizations.

Status of the NRC Nutrient Requirements of Horses

The fifth revision of the National Research Council (NRC) *Nutrient Requirements of Horses* is now 15 years old. Released in May of 1989, it is the most current edition of recommended nutrient allowances for horses. It contains several tables that present nutrient requirements for 19 different classes of horses, based upon age, rate of growth, level of physical activity, age/training interaction, stage of pregnancy and lactation. These tables are replicated for ponies and horses of different expected mature bodyweights of 200, 400, 500, 600, 700, 800 and 900 kg.

The 1989 NRC publication continues to provide a basic framework for ensuring that nutrient requirements of most categories of horses are generally met and not exceeded to the point of toxicity. However, research findings of the past 15 years and fundamental changes in the horse industry must be addressed by a new revision of *Nutrient Requirements of Horses*. To their credit, the NRC has convened an expert committee to review and revise the publication, and the first meeting of this committee is scheduled for April 2004.

Major considerations for the new NRC Equine Committee

Although not an exhaustive list, the following topics
will be important for the new NRC committee to consider during the development of the updated *Nutrient Requirements of Horses*:

- Energy metabolism/requirements
- Carbohydrate metabolism
- Protein/amino acid requirements
- Mineral requirements
- Vitamin requirements
- Water requirements
- Dietary fats and oils
- Geriatric horses
- Feed composition tables
- Bone metabolism/growth
- Dietary/genetic interactions
- Diet/exercise interactions
- Nutraceutical ingredients
- Nutritional/environmental interactions
- Horse owner demands

**ENERGY METABOLISM/REQUIREMENTS**

The energy requirements for most livestock species are given as metabolizable energy (ME), while requirements for horses are currently given as digestible energy (DE). Energy requirements for lactating dairy cattle are given as net energy for lactation (NE\_L). One of the first discussions of the NRC Equine Committee regarding energy requirements might be about the merits of expressing energy requirements for horses of various stages of growth, gestation, lactation or exercise as DE versus ME or NE. An evaluation of various equations proposed in research to estimate energy requirements of horses in various life stages and exercise intensities will be an important consideration of the NRC committee.

**CARBOHYDRATE METABOLISM**

The horse is fundamentally a grazing herbivore that spends as much as 12-16 hrs per day eating forage when on pasture or rangeland. It is a hindgut fermenter with substantial cellulolytic microbial populations in the cecum and large colon. Structural carbohydrates from forage are the most ‘natural’ carbohydrates for the horse to digest with assistance from hindgut microorganisms. However, as the horse is expected to grow rapidly, lactate or perform intense exercise for competition, the ability of structural carbohydrates to meet the horse’s energy requirement is exceeded and starches and sugars from concentrate mixes become necessary. Research has been conducted to evaluate the glycemic responses of horses to nonstructural carbohydrates from various types of grains as well as comparative responses to diets that provide supplemental energy high in starch and sugar versus diets high in fat and fiber. Diets high in starches and sugars have been implicated in numerous metabolic bone problems in growing horses, digestive problems related to acidification of the hindgut, and to laminitis due to toxin production in the hindgut. However, recent studies have confirmed that horses and ponies are quite capable of metabolic adaptation to either high starch and sugar diets or high fat diets (Schmidt *et al.*, 2001; Hoffman *et al.*, 2003).

**PROTEIN/AMINO ACID REQUIREMENTS**

Limited data are available regarding the protein digestibility of specific feed ingredients in horses. Amino acid absorption occurs mainly in the small intestine of the horse, but some ammonia absorption also occurs in the hindgut. Therefore, protein requirements in the horse are expressed as crude protein (CP). Non-protein nitrogen feed ingredients are not efficiently utilized by the horse due to the location of fermentative digestion sites near the end of the gastrointestinal tract. The CP requirement for mature, sedentary horses under maintenance conditions is quite low, at less than 8%. Horses at maintenance can meet CP requirements on moderate-quality forages and grains, but growing horses and especially lactating mares require protein supplementation unless a high-CP forage containing legumes is a major component of the diet.

Amino acid requirements for horses are not well defined, but some work conducted since the publication of the last NRC *Nutrient Requirements of Horses* has suggested that threonine is the second-limiting amino acid (lysine is first-limiting) in a yearling horse diet of corn, oats, soybean meal and coastal bermuda grass/hay (Graham *et al.*, 1994). There is little question that protein quality is of concern for growing horses and lactating mares, and
for the horse in general, as compared to ruminant species. Adequate lysine concentrations should be present in diets high in soybean meal, but diets supplemented with cottonseed, peanut or flax meal could require additional lysine when fed to growing horses or lactating mares. Greater commercial availability and affordability of essential amino acids and advances in plant breeding that alter the amino acid profiles of some feeds are factors to consider when making recommendations about protein/aminio acid recommendations for horse diets. Dietary protein and amino acid recommendations for working horses may some day consider the acidogenic effects of excess dietary protein (Graham-Thiers et al., 2001), although data are still limited in this area.

MINERAL REQUIREMENTS

Mineral concentrations of feedstuffs may vary significantly with soil mineral concentrations, soil fertilization, plant species, harvesting conditions and stage of plant maturity. Special attention should be paid to providing trace mineral supplements formulated specifically for horses, not other species. For instance, trace-mineralized salt for sheep is typically devoid of a bioavailable copper source for horses, since copper oxide is usually the only source of copper. A more bioavailable source should be provided instead. Information on trace mineral bioavailability in the horse is not as extensive as data collected in other species (Ammerman et al., 1995). However, a substantial amount of research has been done in recent years regarding equine mineral nutrition that the new NRC Equine Committee will need to evaluate. Calcium and phosphorus status of equine bone has been found to be related to physical activity as much as dietary status. Extreme variations in the Ca:P ratio of different forages have a great impact on the composition of supplements recommended to complement the basic forage ration. Trace mineral nutrition has received substantial attention from researchers in recent years, and mixed results have been obtained regarding the relative bioavailability of different trace minerals fed as inorganic versus ‘organic’ forms of trace minerals that are bound to an organic molecule.

VITAMIN REQUIREMENTS

Fresh green forages and exposure to sunlight are all that is necessary to meet the vitamin needs of most horses. Horses under confinement conditions may lack one or both of these factors, however. Forages preserved with propionic acid or stored for extended periods of time may lose much of their vitamin A activity. It is not unusual for show horses or for horses being prepared for sale to be housed indoors for extended periods of time during summer daylight hours in order to avoid dulling of the hair coat from the sun. These horses may lack vitamin D if good-quality, sun-cured forage is not available. Vitamin E could also potentially be deficient if poor-quality forages are fed for extended periods.

B-complex vitamins are synthesized in the horse’s hindgut and usually do not require supplementation. Although no dietary biotin requirement has been established beyond what is synthesized in the gut, research continues into the possible use of supplemental biotin for improving poor-quality hooves. Two studies have found improved hoof quality in horses with initially poor hoof quality when fed 5-10 mg of supplemental biotin per 100 kg bodyweight per day for periods ranging from one to six years (Josseck et al., 1995; Geyer and Schulze, 1994). Another study found a 15% increase in the rate of hoof growth in ponies with 0.12 mg/kg bodyweight of biotin as compared to ponies on a control diet without supplemental biotin (Reilly et al., 1998). Feeding recommendations for vitamin E could be viewed both in terms of requirement levels needed to avoid deficiency symptoms and also as optimal levels for minimizing exercise-induced oxidative damage to cells through free-radical formation and for maximizing immune function.

WATER REQUIREMENTS

A series of recent studies at Michigan State University (Butudom et al., 2002; 2004) found that horses dehydrated by either exercise or frusemide administration drank more fluid when presented as either 0.45% NaCl or 0.9% NaCl solutions as compared to regular water. Their work also supported previous observations that horses quench their thirst faster and delay rehydration when consuming cold fluids after dehydration as compared to warm fluids, and that the primary stimulus of thirst is an increase in plasma tonicity rather than hypovolemia (Butudom et al., 2003). Horses consumed more 20°C water than 10°C water after being dehydrated. Geor et al. (1998) found that pre-exercise hyperhydration provided no thermoregulatory advantage to horses and Sosa et
al. (2002) found that hyperhydration using an isotonic solution at 6% of body weight resulted in arterial hypoxemia, possibly from pulmonary edema. These recent studies and others will likely help to guide the NRC Equine Committee’s recommendations regarding provision of electrolyte solutions versus water for rehydration of already dehydrated horses.

**DIETARY FATS AND OILS**

The use of dietary fats and oils has been one of the most active areas of research since the publication of the fifth revision of the National Research Council (NRC) *Nutrient Requirements of Horses*. Horses can utilize fats and oils effectively, and inclusion rates of up to 15% of the concentrate ration may be well accepted by the horse. One study found that the jejunal microflora of ponies was not affected by the addition of up to 20% coconut oil in the diet (Kollarczik et al., 1995). Research has suggested that fats and oils in equine diets may provide benefits to horses in addition to increased caloric density of the diet for weight gain or maintenance in 'hard keepers'. Dietary fats and oils may also help to improve hair coat quality, spare muscle glycogen in exercising horses, reduce post-exercise lactic acid accumulation, and reduce the chance of enterotoxemia, colic and founder. Not all studies have found beneficial effects, however. Excess fats and oils reaching the hindgut may inhibit fiber digestion (Jansen et al., 2000) and diets high in fat and fiber when fed to growing foals may reduce the absorption of calcium and other minerals necessary for bone development (Hoffman et al., 1999). The preponderance of research data, however, appears to support the continued expanded use of dietary fats and oils for many classes of equines. Research suggesting that added dietary fat may reduce the heat production of performance horses working under hot and humid conditions (Kronfeld, 1996), and enhance the oxidative capacity of muscle (Orme et al., 1997) continues to stimulate interest in this dietary supplement for horses.

**GERIATRIC HORSES**

With more and more elderly horses represented in the equine population every year, and more commercial diets being formulated for geriatric horses, this area of research seems to be a promising one for equine nutritionists, yet little work has been done in determining nutrient requirements and feeding management strategies of geriatric horses. This is most likely due to the fact that so many different age-related infirmities may be related to poor nutritional performance in geriatric horses. Poor teeth, malabsorption, endocrine problems such as Cushing’s disease, chronic pain, etc. may all affect digestion, absorption, and metabolism of nutrients. Such variability among geriatric horses makes controlled study of this group of animals difficult. The typical feed formulation strategies being employed by feed manufacturers include processing of both forages and concentrates to improve foregut digestion of starches and improving hindgut digestion with highly fermentable fiber like beet pulp and decreasing particle size of fiber to enhance hindgut fermentation. Addition of microbial products to further enhance hindgut digestion and adding fats and oils to improve caloric density have also been employed. There is opportunity for further research in this field.

**FEED COMPOSITION TABLES**

As novel by-product feeds, genetically modified plants and new crops are added as potential livestock dietary components, the NRC Equine Committee will need to review those products that may have an application to equine diets. Significant modification of the NRC feed composition tables will likely be necessary.

**BONE METABOLISM/GROWTH**

Glade (1993) found that mid-cannon bone diameters of foals born to mares fed calcium-deficient diets were thinner and mechanically weaker at birth than in foals born to mares fed NRC recommended concentrations of dietary calcium. These differences in limb bone size and strength persisted during the first 40 weeks after birth. Hoffman et al. (1999) found that bone mineral content was lower in weanlings fed a supplement high in fat and fiber compared to weanlings fed a supplement high in starch and sugar, and they speculated that “binding of calcium by fat and fiber may alter the availability of elements necessary for bone development.” Studies comparing diet manipulations and evaluating the resulting changes in serum skeletal metabolic markers such as hydroxyproline (bone resorption), osteocalcin (bone mineralization), C-propeptide of type-II procollagen (collagen synthesis) as well as
measurements of the physical properties of bone promise to further elucidate the effects of diet on bone metabolism and growth in horses.

**DIETARY/GENETIC INTERACTIONS**

Although feeding programs designed for horses with genetic defects should never find a large market, there has been substantial interest in managing some of the more common equine metabolic disorders with diet. The common genetic defect manifested as hyperkalemic periodic paralysis (HYPP) in Quarter Horses has been managed with both medication and low potassium diets. Polack et al. (2000) found that horses with equine motor neuron disease had greater concentrations of copper and lower concentrations of vitamin E in the spinal cord, suggesting that an imbalance of oxidant/antioxidant nutrients may have an effect on the occurrence of this poorly understood disease. Recurrent exertional rhabdomyolysis (RER) has been associated with a diet high in soluble carbohydrate. McKenzie et al. (2003) found that horses severely affected with RER that were fed a high-fat, low-starch diet had dramatically lower post-exercise serum creatine kinase (CK) activity compared to horses fed a low-fat, high-starch diet. Firshman et al. (2003) found that polysaccharide storage myopathy (PSSM) in Quarter Horses can be managed by following dietary recommendations of reduced soluble carbohydrates and supplemental fats and oils combined with gradual increases in daily exercise.

**DIET/EXERCISE INTERACTIONS**

The NRC Equine Committee will have numerous studies to review related to the energetics of exercising horses. Many studies, though, deal with more than meeting additional energy demands. Researchers have shown great interest in evaluating diets that may help exercising horses to maintain proper immune function, enhance aerobic metabolism of fatty acids and minimize lactate production from anaerobic glycolysis. The timing of meal feeding with respect to exercise has received a great deal of attention in recent years. St. Lawrence et al. (2002) found that 48 min of light work performed 1 hr before a meal did not affect glucose or insulin responses to the meal. Jose-Cunilleras et al. (2002) found that feeding a soluble-carbohydrate-rich meal (corn) to horses before exercise increased muscle utilization of blood-borne glucose and carbohydrate oxidation and decreased lipid oxidation compared with a meal of insoluble carbohydrate (alfalfa) or not feeding. Carbohydrate feedings did not produce a sparing of muscle glycogen compared with fasting. Pagan and Harris (1999) found that feeding grain before exercise reduced free fatty acid (FFA) availability and increased blood glucose disappearance during exercise in Thoroughbred horses. Feeding hay either along with grain or ad libitum the night before exercise resulted in reduced plasma volume and higher lactate production and heart rates during exercise. They concluded that feeding only forage before exercise did not adversely affect performance. They also suggested that grain should be withheld from horses before exercise, but that small quantities of hay should be fed to ensure proper gastrointestinal tract function.

**NUTRACEUTICALS**

The term nutraceutical was coined in the 1990s by Dr. Stephen DeFelice in reference to dietary substances affecting human health. The term has gained popularity in reference to dietary products that may impart health benefits to horses as well. Dr. DeFelice defined a nutraceutical as:

“...any substance that is a food or a part of a food and provides medical or health benefits, including the prevention and treatment of disease. Such products may range from isolated nutrients, dietary supplements and specific diets to genetically engineered designer foods, herbal products, and processed foods such as cereals, soups and beverages. It is important to note that this definition applies to all categories of food and parts of food, ranging from dietary supplements such as folic acid, used for the prevention of spina bifida, to chicken soup, taken to lessen the discomfort of the common cold. This definition also includes a bio-engineered designer vegetable food, rich in antioxidant ingredients, and a stimulant functional food or pharmafood.”

As the number of geriatric horses increases, and as horses compete in more demanding competitions, the interest among horse owners in such nutraceutical products is increasing. Documentation of benefits of nutraceuticals in horse diets has been very limited. Orth et al. (2002) found that the combination of glucosamine and chondroitin sulfate inhibited the synthesis of several mediators of cartilage degradation...
in equine carpal cartilage explants in vitro. Evidence of benefits to equine joint health from supplementation with glucosamine and chondroitin sulfate in vivo has been inconclusive.

NUTRITION/ENVIRONMENTAL INTERACTIONS

Horses are frequently housed in facilities that are designed more for the comfort of the horse owner than for the horse. Horse housing is notoriously poorly ventilated to the point of being airtight in some cases, and horses are forced to breathe poor-quality air. The problem is exacerbated when horses consume diets containing excessive protein, resulting in high rates of urea excretion and high aerial ammonia concentrations. Fear of fecal contamination of hay and/or grain with the causative organism for Equine Protozoal Myelitis has prompted some horse farm managers to feed all forages and concentrates entirely as bagged cubes, pellets or texturized feeds. Hay cubes, complete hay/grain cubes and completely pelleted diets are alternative methods for providing forage for horses. The primary motivation for using these forms of feed is the extreme ease of storage and handling under confinement situations or when frequent hauling of animals precludes transport of bulky-type feeds. Because nutrient levels in complete feeds can be precisely defined while still providing adequate roughage, they are gaining favor as creep rations for rapidly growing foals and even as high-performance race horse diets as well as diets for geriatric horses. Hay supplies tend to be unpredictable at racetracks, which makes ration balancing difficult. The potential for increased wood chewing, boredom, colic or founder of horses on complete feeds has been cited as being of concern. However, trainers using complete hay-grain feeds have reported that horses eat the complete pellets slower than grain alone, and that boredom and health problems have not increased. Research on a complete cubed feed tends to support these observations (Younglove et al., 1994). Environmental stress, intense exercise training, large amounts of concentrated high-energy feeds, small rations of forage and a low feeding frequency per day are associated with gastric ulcers in horses (Feige et al., 2002).

Another consideration related to nutrition and the horse’s environment has to do with management of excess nutrients in fecal and urinary waste. Excess nitrogen, phosphorus, trace minerals such as copper and zinc and other nutrients fed in excess may be subject to greater scrutiny and governmental regulation of waste management in the future. Further precision in defining nutrient requirements of horses may help to minimize unintended environmental impacts due to excess provision of certain nutrients.

HORSE OWNER DEMANDS

The psychology of feed buying decisions by horse owners is not the primary focus of the NRC committee, but this consideration is a significant factor in determining feeding strategies, feed forms and nutritional products that horse owners are willing to offer their horses. The adequacy of dietary energy, protein, vitamins and minerals in a horse’s diet may receive considerably less conscious thought from the horse owner than the expression of diet sufficiency as a glossy hair coat, vigorous attitude, hearty appetite and soundness of limb and wind. The extent to which today’s horse owner wants her horse to be not merely healthy, but also fully contented, can be a great motivating force that drives feed-buying decisions. This general mind-set of many modern horse owners tends to drive the demand for horse feeds that are both high in nutritional quality and in sensory satisfaction to the horse and owner. The appearance, texture, aroma, ease of handling and palatability (from the standpoint of the horse’s apparent enjoyment) are major considerations when buying feed. The value of a commercial horse feed has as much to do with serving the horse owner’s emotional needs as providing adequate nutrient content in a cost-effective manner. Successful feed companies have developed profitable new product lines based upon identifying the motivations behind the buying decisions of horse owners. Today’s horse owner has shown a tremendous willingness to pay a premium price for high-quality products that directly acknowledge their view of the horse as both a livestock equine partner and a companion.

References


Introduction

A strong, well-conformed and developed musculo-skeletal system is essential to provide structural support and limb soundness in exercising horses of all ages. These needs are greatest in the athletic horse to withstand the loading, strain and concussive forces imposed on bones and joint structures. Bone and joint injuries account for up to 70% of the downtime or lost training days in racing and equestrian event horses, with the type of musculo-skeletal injury changing during a horse’s athletic career (Bailey, 1998).

The lower forelimb below the knee of horses is most likely to develop structural damage leading to lameness in all classes of athletic horses (Davies, 2003). Over the past decade, there have been numerous studies that have provided insight into the reactive properties of bone and limb structures as they adapt to the increase in body weight and exercise loading from birth and throughout a horse’s life (Lawrence, 2003b).

Musculo-skeletal unsoundness, particularly related to bone failure and joint injury in racing, equestrian and other athletic horses, can be linked to overloading of bone structures relative to the body weight of the horse, the age at which the young horse is first worked, the speed of exercise and degree by which the bone and joint structures are able to adapt over time to additional body weight and loading forces (Ireland, 1998; Davies, 2001). After each period of exercise, even in growing horses, the bones remodel or react to increase strength, circumference and mineral density (collectively called bone mass) while internal trabecular structures adapt to withstand the increased loading forces. This is a relatively slow process, which can take up to four months to complete in a progressive remodeling process in the young horse exercising during the growth phase or in athletic training before maturity (Lawrence, 2003b).

If the increase in loading force exceeds the rate at which the cross-sectional area and mineral density of cortical bone can adapt, then there is a risk of bone reaction. This results in bone surface (periosteal) inflammation; and in bone subjected to the repeated stress of high loading, there is the risk of bone shaft and joint microfracture and failure (Davies, 2001).

One of the classic examples of the failure of bone modeling to increase bone mass and resilience at an optimum rate in response to exercise loading is dorsal metacarpal disease, commonly known as shin soreness or ‘bucked shins’ in young Thoroughbred racehorses. (Bailey, 1998; Lawrence, 2003b; Davies, 2003). A similar overloading-induced reaction and metacarpal fracture also affects the metacarpals of racing greyhounds, referred to as metacarpal periostitis, when dogs are exercised on a ‘too fast – too early’ program on a tight turn racetrack (Boemo, 1998; Ireland 1998).

The highest weight loading is imposed on the bone structure of the front limbs of racing Thoroughbreds when cornering at the gallop at speeds of up to 15 meters/second (Bailey, 1998). Studies have shown that loading forces on the front limbs of up to twice that of a horse’s bodyweight are imposed when galloping in a straight line (Lawrence, 2003b). For example, when a horse is galloped around a corner on a racetrack, an estimated combined centrifugal and momentum-related loading force of up to 5-10 times the animal’s body weight is placed on bone and joint structures in the lower limbs (Ireland, 1998). Up to 80% of lameness conditions occur in the front limbs of galloping racehorses, with up to 80% of these injuries and failures or ‘breakdowns’ focused on the bones, joints, ligaments and tendons below the knee (Bailey. 1998). Failure of the musculo-skeletal system is most often associated with injury.
to the hard tissue or bone structures of the lower limb, with tendons and joints also being subjected to ‘wear and tear’ and overload in over-galloped young horses (Lawrence, 2003b).

**Bone development in growing horses**

Bone is a dynamic living tissue that is responsive to the loading forces imposed during exercise from birth, with most response occurring in the first six months of life. A number of studies have indicated that joint diseases or the failure of proper joint and associated bone development known as osteochondrosis, which refers to a primary lesion or defect in the growth and maturation of cartilage and subchondral bone, is most common as cartilage changes to bone as a foal grows (Jeffcott, 2001; Firth, 2003a; Lawrence, 2003a). The mineralization of the cartilaginous skeletal bone structures commences in the unborn foal during the last three months of gestation as it doubles in size prior to birth (Jeffcott, 2001).

At birth, the foal’s skeleton contains only 17% of the mature bone mineral content, increasing to 68.5% by six months of age, and 76% by yearling age in athletic breeds of horses (Lawrence, 2003a; Firth, 2003a). Studies have shown that cartilage defects occur within the cartilage of the joint surface or at the epiphyseal junction, or growth plate on the ends of the long bones, as the skeleton develops in the growing horse.

The immature bone is composed mainly of cortical or external wall or compact bone, and cancellous or porous, trabecular reinforcing bone within the bone’s internal structure (Lawrence, 2003a). Mineralization of cortical bone with deposition of calcium, phosphorus and magnesium, the principal bone minerals, continues as the body weight and exercise loading increases throughout the first year of life. Subchondral bone and structural shaft bone must continually adapt, strengthen by additional mineralization and repair themselves when subjected to loading, compression and concussion during exercise as the skeletal system develops in the young horse. After each period of exercise, the bones react to remodel or adapt their internal structure and strength by increasing the cortical cross-section, circumference and mineral density (Lawrence, 2003b; Davies, 2001).

The area of cortical or shaft bone increases from around 30% at birth to 60% at six months to 80% of the skeletal bone structure in the mature horse (Firth, 2003b). Davies (2001) describes the remodeling and changes in cortical shape of bone in horses as they progress through a training program. A technique of Radiograph Index to measure the cortical thickness of the front cannon bones has been developed and is used to assess the state of remodeling relative to the stage of training in young Thoroughbred horses, and may be used to predict the extent of bone reaction and shin soreness (Davies, 2003).

**Bone adaptation to exercise**

One of the earliest studies in America on Thoroughbred and Quarterhorse weanlings concluded that bone strengthens by increasing its mineral density, principally by depositing calcium within bones (Raub et al., 1989). These researchers found that over an 111-day period the cannon bones of weanlings exercised by trotting, initially over 400 meters and increasing to over 4 km per day, accumulated 25% more bone calcium than weanlings that had been stalled overnight and turned out into pens during the day.

Numerous other studies, cited by Jeffcott (2001), Firth (2003a) and Lawrence (2003b) in a review of osteochondrosis and response to exercise in horses, have reported similar adaptive responses in foals and weanlings up to five months of age, with a lower bone density in the cortical shaft and subchondral bone mass in non-exercised compared to exercised young horses. After six months of identical exercise, the young horses in the original non-exercised group remodeled the bone, in this case the stifle joint, to establish an equal mineral density and cross-sectional area (Lawrence, 2003b).

The mineralisation of the skeletal and the structural subchondral base of joint cartilage, as well as the calcification of long bones and formation and internal organization of the tendon structure, is largely completed by six months of age. It has been established that the amount and type of exercise has a direct influence on joint cartilage, subchondral bone, bone cortical mass and maturation in young horses as they develop.

It is important that young growing horses have access to free paddock exercise to encourage the formation of sound cartilage and subchondral bone, while over-exercise and excessive weight loading in heavy weight young horses can result in damage to the developing joint cartilage and subchondral bone in joints (Firth, 2003a).

Jeffcott (2001) and Firth (2003a) concluded that the effects of high energy:nutrient ratios, confinement
to small yards and over-exercise or lack of adequate exercise, high weight loading and inadequate trace minerals and calcium balance, all adversely influence the cartilage development, bone mineralisation and maturation of the skeleton in the formative years of a horse’s life.

INCREASED BONE TURNOVER

One of the other major problems in bone repair is triggered by the stress of everyday training. The bones must continually adapt and remodel themselves to maintain strength in response to training. The stress of continuous high loading results in the bone itself becoming less dense during this modeling process, triggered in response to ongoing high load exercise. During each hard workout, the high stress loading on the bones can cause microfractures or other microscopic changes within the cortical bone, ultimately causing a repair response within the bone (Davies, 2001).

BONE STRESS CONDITIONS

Studies of shin soreness in Thoroughbred racehorses have shown that after a hard gallop over 200-300 meters (1 – 1½ furlongs), the cannon bone in a young horse becomes reactive and attempts to strengthen itself by depositing calcium within its front cortical wall for the next 10-12 days (Nunamaker et al., 1990; Davies, 2001). When too many ‘breeze-up’ or ‘all-out’ gallops are given successively at 2-3 day intervals in an accelerated ‘get fit’ or ‘too fast – too early’ training program, the bone itself cannot respond rapidly enough, and can become inflamed, as occurs in shin soreness (Davies, 2001).

Once horses reach maturity at four years of age, the bone remodeling process is less active. Studies have shown that in the young horse, an injury to a bone can affect the response within 2-5 days, but in an older horse this may take up to 10-12 days, depending on the type and extent of the exercise overloading (Firth, 2003b).

ABNORMAL BONE TURNOVER

In response to high loading forces, the bone needs to remodel to either repair itself, or to strengthen the target site where the overload has occurred. In the athletic horse, there are a number of sites that are more likely to fracture when working because of abnormal bone turnover during exercise. Studies using radio-isotope labeling of bone calcium and other minerals, as well as traditional radiographs of the bones, can help monitor the structure and density of the bone. Recent developments, such as scintigraphy of the bone, can map the responsive nature of bone and identify the sites where active remodeling and calcium deposition is occurring as new bone is formed. These studies have shown that certain bones in the horse’s skeleton undergo a high rate of repair and remodeling during training. If this rate of remodeling is unable to be maintained to strengthen and rebuild bone in response to exercise, the bone itself is more likely to fracture and fail when loaded. These include the edges of the pedal bone in the hooves, the sesamoid bones behind the fetlocks and the knee and hock bones in racing and hard working horses (Firth, 2003b).

ADAPTATION TO FAST EXERCISE

Studies have investigated the effects of exercise on the bone density and strength in growing and adult horses at both training and racing intensities of exercise. It has been revealed that when a horse is worked below its maximum speed during long periods, in both young and adult horses, there is no dramatic alteration in the density or mass of bone in the front cannon bones (Lawrence, 2003b). However, once horses start galloping at a much faster speed in fast work, there is an increase of bone density relative to the speed of exercise, and a reduction in the porous structure of the bone as more calcium is loaded into the bone to strengthen it in response to exercise (Davies, 2001).

DORSAL METACARPAL DISEASE (SHIN SORENESS OR BUCKED SHINS)

Dorsal metacarpal disease, or ‘bucked shins’ in the severe case, is a common example of bone overload and associated bone reaction. Thoroughbred trainers in the United States, Australia and New Zealand often have young horses that develop dorsal metacarpal disease. It is a widespread belief held by many trainers in Australia that young Thoroughbreds in early race training need to develop shin soreness to ‘toughen’ their cannon bones (Bailey, 1998). This condition, however, is a symptom of excessive bone loading resulting from a program of too fast-too early pace work or breeze-ups.
Although much research has been carried out investigating the classic changes that occur (Nunamaker et al., 1990; Davies, 2001), many researchers fail to understand the relationship between speed and radius of a circular training track. Ireland (1998) highlights the relationship between body weight, speed and radius of the corner on a race track that influences the rate of injuries and incidence of metacarpal periostitis in young greyhounds analogous to shin soreness in horses. Ireland (1998) provides evidence of a direct relationship of these factors to the degree of centrifugal force loading that occurs when a horse or greyhound sprints around a bend on a racetrack. When the track surface has an adequate crossfall to the inside rail or is banked in proportion to the speed of movement and radius of the track corner, the horse is able to lean over to be perpendicular to the track slope and the centrifugal force is negated, reducing its high loading forces on the limbs (Ireland, 1998).

Galloping in a straight line will not induce periosteal reaction to the same degree as occurs when the cannon bones are loading as a horse is galloped around a compacted, inadequately banked bend on the racetrack. In Australia and the southern hemisphere, dry compacted track surfaces that increase limb concussion combined with over-galloping young horses on circle tracks are the major contributing causes of forelimb metacarpal periostitis and shin soreness (Bailey, 1998). Shin soreness in young horses can vary in severity, but it describes the inflammation and pain that develops over the front surface of the foreleg cannon bones in young racehorses galloping at racing speed. A much better understanding of bone remodeling processes has led to revised training and management guidelines to reduce the risk of it developing in young racehorses.

**Incidence**

Surveys indicate that the risk of metacarpal remodeling in horses (and greyhounds) is influenced by the track surface (moisture content and compaction) and design (banking and length of straight gallop), radius and crossfall of bends and end circles, seasonal conditions and the speed and distance of fast exercise in the training program (Ireland, 1998; Bailey, 1998; Table 1).

The leading foreleg has a 20% higher risk of developing shin soreness, although both front cannons are affected in 70% of young horses (Bailey, 1998). Colts and fillies are affected equally. The direction of cornering also influences the severity of shin soreness on the inside limb next to the running rail, with the leading inside foreleg cannon bone developing a more severe periosteal inflammatory response and remodeling reaction. The cannon bones must change their cross-sectional shape to provide reinforcement along the stress pathways imposed by high speed exercise (Davies, 2001). It is a slow natural process that must happen to enable the bones to withstand high-speed strain forces without developing stress related microfractures that radiate into the anterior cortex of the cannon bones.

**Underlying causes**

Dorsal metacarpal disease has been shown to be initiated by increased strain forces from concussion, increased weight forces and centrifugal turning forces in horses exercising at racing speeds on tracks with unbanked, small radius bends (Ireland, 1998). The cannon bones change in shape and diameter as the horse adapts to training (Ireland, 1998; Davies, 2001). Very high bone strain, compression and cyclic loading forces in the front third metacarpal (shin) bones have been recorded in early training (Davies, 2001).

When a young horse is worked below maximum speed for extended periods, there is no dramatic alteration in the density or mass in the front cannon bones (Davies, 2001). Generally the risk of shin soreness can be reduced by adopting a revised training

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**Table 1. Incidence and reasons for occurrence of dorsal metacarpal disease in the UK, US and Australia.**

<table>
<thead>
<tr>
<th>Incidence (%)</th>
<th>Training influences</th>
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<tr>
<td><strong>United Kingdom</strong></td>
<td>9 – 17</td>
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<td><strong>United States</strong></td>
<td>65 – 70</td>
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1 Bailey, 1998
program allowing the cannon bones to be challenged with controlled strain loads by galloping over short distances at an earlier stage of training (Nunamaker et al., 1990; Boston and Nunamaker, 2000). This incremental loading pattern allows the bone to undergo the required remodeling changes with more time to adapt to fast work at racing speeds. If a horse is pushed too fast too early, the stress loading on the immature bone stimulates emergency modeling with deposition of weaker fibrous elastic bone to reinforce the bone so that it can withstand the forces of galloping (Davies, 2001).

Centrifugal force (Cf) or loading on the cannon bone is related to the following equation (Ireland, 1998):

\[ Cf \approx (BW \text{ of horse + rider}) \times \text{Speed}^2 \]

Radius of the track end circle

This equation illustrates that heavy, faster 2-year olds galloping at racing speeds on unbanked, compacted small tight circle tracks have a higher risk of bone overloading and bone reaction.

When a horse is turned out and rested in the paddock after going acutely shin sore, with shortened stride, swollen, painful ‘bucked shins’ on the anterior surface of the forelimb cannon bones, the weak fibrous bone is resorbed, returning the bone to its immature cross section and lower load bearing capacity over a period of 4-8 weeks (Boston and Nunamaker, 2000).

Ideally, the front cannon bones should be encouraged to adapt slowly from the start of training, allowing accumulation of extra high density mineralised cortical bone to strengthen the anterior surface of the cannon bones to enable them to withstand concussion and increased weight bearing loads. Rapid reinforcement with fibrous bone involves the reabsorption of calcium from the sides to strengthen the front surface, reducing the bone’s side rigidity or ‘stiffness’ against bending under high strain loads. This can result in painful stress fractures developing on the anterior surface of the front cannon bones as they distort under extreme loading forces of high speed galloping and symptoms of ‘bucked shins’ and risk of metacarpal fracture and failure during racing (Nunamaker et al., 1990).

**Practical management for prevention**

Based on my 30 years of experience as an equine veterinarian and my professional role as a consultant to many racehorse trainers in Australia, I have developed a management program which can be implemented by race trainers to significantly reduce the risk of bucked shins, or assist the rehabilitation of horses that have developed severe periosteal inflammation and cortical microfractures. This program is based on the research outlined in this review and is intended as a practical guide.

**GENERAL INFORMATION**

Most Thoroughbred racehorses train at speeds much slower than those at which they need to race to be competitive, where they can reach peak speeds of up to 50 km/hr (15 meters/second). Therefore, a horse may be modeling its cannons to withstand the loading imposed by slower conditioning, which is much lower than the strains imposed at racing speed. Thus, when the horse actually undergoes prolonged fast work, such as a barrier trial or its first race, the risk of sudden and intense bone overload and damage is increased, especially if it is galloped at speed around a tight circle track after being trained on a relatively straight track. The cannon bone has to model from a circular, even-walled cross-section in the immature horse to a cross-section with a thickened inside edge, with up to twice the average wall (cortical bone) thickness of the cannon bone shaft.

A technique of radiographic index (RI) with radiographs taken to measure the thickness of the bone wall (cortical bone) on the front and inside edge of the front cannon bones at weekly intervals can help monitor the adequacy of the remodeling process in response to bone loading during training. If the RI exceeds the established value at a set stage of training, it is an indication that excessive bone remodeling and inflammatory reaction has occurred. In this case, the speed and distance of fast work should be reduced to allow the remodeling process to catch up to the degree of strain loading. If the RI is below the established limits at a particular stage of training, it is possible that horses that are over-galloped have a high risk of developing shin soreness.

Many trainers believe in long, slow work to ‘build bone’. While this program works to build and condition the muscles, joints and limbs, the bone that the horse is building is more suited to long, slow work rather than to racing. If the shins become severely inflamed with deposition of a bone splint or a ‘bucked shin’ swelling, the bone laid down is a weaker form of fibroelastic bone as a temporary reinforcement to prevent bone failure. This type of bone has little residual strength; and if the horse is
rested from training, this temporary reinforcement 'splint' is resorbed. The bone profile or cross-section returns to its original weaker structure that is unable to withstand the increased loading imposed by galloping too fast-too early when training resumes.

Harness horses do not develop sore shins because they race at slower speeds on banked tracks and have two legs on the track surface at any one time to share the additional loading.

Training programs should be designed to allow the cannon bones to experience and adapt to the loading and deformation strain imposed by higher speed galloping. The leading forelimb is subjected to the greatest amount of strain, particularly when cornering, so it is recommended that the initial high speed work should be carried out down the straight to model the shins, reducing to a slower pace around the turns.

**Short gallops in early training**

Commence a program of short, straight-line gallops over 200 meters twice weekly (at least 4-5 days apart) from the third week of training to progressively load the cannon bones. Short gallops in a straight line do not cause undue stress on bones or muscles after 2-3 weeks of initial conditioning exercise. As the horse develops in fitness, increase the speed and distance traveled to 300-400 metres at each fast workout. Bone modeling is a slow process as is bone fracture healing, so a stepwise increase in loading over 8-10 weeks will allow time and stimulate modeling in response to the loading forces imposed. A measurement of Radiographic Index to evaluate the remodeling response of the bone can be carried out at weekly intervals to monitor the bone thickness in response to fast work.

**Avoid tight circle tracks**

Do not gallop young horses around tight, compacted curves or end circles on the track too early in their training preparation. Gallop only up straights initially, then work progressively faster into corners and around end circles to allow adaptation to the centrifugal sideways strain forces, starting after 6-8 weeks of training. Avoid sudden introduction to fast work and limit speed of galloping around unbanked, relatively tight bends initially, especially on dry, compacted tracks.

**Adequate calcium and vitamin D**

Ensure high grain diets are supplemented with calcium, phosphorus, magnesium, vitamin A and vitamin D to provide adequate minerals for bone development and modeling in response to loading as the shin bones model as they thicken and adapt in cross sectional shape to withstand the increased stress loads.

**Train on the same track**

It is best to train on the same track, so that the bends and surfaces are consistent, avoiding compacted areas on the rails when galloping on fast work mornings. The majority of racetracks have training tracks built inside the main grass, turf surface or sand track. These have smaller end circles and can impose additional loading, especially on odd shaped, rather than oval track designs, constructed to provide the length of straight required within the available land area.

**Avoid heavy work riders**

Once the young horse is controllable, change to a fast work rider (50-60 kg) to reduce excessive loading as the horse is worked faster at the start of regular fast work. Each extra kg of rider weight is magnified 5-10 times by centrifugal forces when galloping around bends.

**Minimise concussion**

Cushion depth and type of surface is important to reduce concussion, vibration and ‘jarring’ on the front cannon bones. Properly maintained synthetic, woodchip and grass tracks are less concussive on the legs than dry finely packed sand surfaces. Avoid hard compacted cinder or dirt tracks with tight bends and little banking. Work out from the compacted areas away from the rails and choose dampened, more cushioned areas, if possible, for galloping.

**Cold therapy after galloping**

Apply cold therapy, such as cold water hosing for 10-15 minutes, or preferably by ice boots or an ice block under a pressure bandage for 5-10 minutes, after each gallop in the later stages of training. Ice packs can be wrapped on both front cannons during the walk to cool-out, or during the trip home from the track to help reduce minor inflammation and soreness as training progresses. A magnetic bandage with 1500 gauss field strength wrapped on both forelimb cannon bones may be helpful to increase the rate of deposition of calcium and bone minerals and hasten the modeling and strength of bone laid down as
it adapts to higher loading during fast work. The bandage can be wrapped in the afternoon and overnight when the horse is resting in its stable or yard.

**Check shins regularly for heat or soreness**

Watch for abnormalities in gait and shortening of stride. If early soreness is detected, cut back on the speed of fast work for 2-3 weeks to allow the shins adequate time to model and thicken the bone along the stress pathways. Apply cold packs after training, or if discomfort is more severe, apply a warming liniment overnight or alternatively (not at the same time) a clay poultice overnight until symptoms subside. In severe cases, it is best to rest the horse for 3-4 weeks until the soreness settles down, and then recommence on a revised program as outlined above. When horses are rested, the cannon bone actually becomes even less rigid as modeling of new bone occurs along the stress lines.

**Re-program training after down time**

For each week that a young horse is rested because of other problems (e.g. respiratory disease, joint problems, severe tying-up), back step its training program by two weeks to avoid shin soreness as the cannon bones may start to resorb calcium and weaken during the lighter work period.

**Compounding feeds to promote skeletal development**

The requirement for a balanced and well formulated diet is paramount to ensure optimum growth and skeletal development, as well as remodeling in response to exercise, in young horses in their formative years. It is important that feeds are formulated to provide adequate digestible nutrients to meet these requirements. There is a wealth of information and nutrient recommendations from equine nutritional research over the past two decades. Most companies that produce blended feeds, or large horse breeding farms and training stables are provided with up-to-date advice by equine specialists.

**RATION FORMULATION FOR GROWTH AND DEVELOPMENT PHASES**

It is important that rations are formulated to meet the various phases of reproduction in mares. It is particularly important in the last trimester that pregnant mares be provided with an adequate amount and balance of macrominerals, trace minerals and vitamins to ensure the formation of cartilage and bone framework prior to birth in the unborn foal. It is important that trace minerals such as iron, copper, zinc, manganese and vitamins including vitamins A, D and E are supplemented or incorporated into feed mixes to allow adequate foetal liver storage prior to birth.

After birth, during the more rapid phase of growth, a low glycaemic diet should be provided, containing adequate energy and quality protein as well as minerals, trace minerals and vitamins to meet the growth and mineral needs of the developing musculoskeletal structure. It is beyond the scope of this paper to provide specific guidelines. However, there are a number of aspects of feed formulation that should be kept in mind when blending feeds, premixes and supplement concentrates for use in growing and exercising horses. These issues are rarely addressed by formulators or advisory consultants. These include:

**Limit the concentration of alkaline mineral sources.**

Limit calcium carbonate, magnesium oxide and potassium chloride in the fines of a textured feed, premix or supplement which also contains trace minerals and vitamins. In textured feeds, calcium carbonate is commonly included in the fines at a rate of 1-3% by weight to provide calcium. In premixes and supplement concentrates, it is often used as the carrier at 50-80% of the total batch weight. The alkalinity of these carriers in the fines or bases can reduce the bioavailability and accelerate the degradation rate of vitamins surrounded by an alkaline environment, particularly in a moist feed blend or premix. The majority of essential vitamins, including retinol, cholecalciferol and tocopherols, thiamin, riboflavin and cyanocobalamin are more stable when the pH is maintained between 5-6 in the fines and premix carrier. Compounding a range of vitamins into a pelleted form for blending with alkaline carriers would significantly reduce the destructive interactions during storage. Overages of vitamins should be included to counteract the destructive interactions when these alkaline carriers are used. (Kohnke, 2002).

The addition of calcium hydrogen phosphate, also known as dicalcium phosphate (DCP), an acidic mineral source (23-25% elemental calcium, 18% elemental phosphorus) as a dual calcium and
phosphorus source, blended in a 60% dicalcium phosphate to 40% calcium carbonate ratio, relative to the mineral balance required, will maintain a more neutral pH environment to improve vitamin stability. Calcium carbonate (limestone carrier) has a pH of 8.41 (alkaline) when measured using 10 g in 20 ml distilled water while dicalcium phosphate has a pH of 6.68 (slightly acidic). A blend of 60% limestone with 40% dicalcium phosphate (12 g + 8 g in 15 mls distilled water) has a pH of 6.83. The use of buffered propionic acid mould inhibitors, such as Mold-Zap™ (Alltech Inc.) at standard addition rates, also helps to buffer the alkalinity of carriers to help improve vitamin stability (Kohnke, 2002; 2003, unpublished). In addition, calcium is absorbed more effectively from the small intestine in an acidified environment.

Mineral oxides, particularly iron oxide as well as copper oxide and zinc oxide, should be limited in premix blends

Oxides generally reduce vitamin stability. Magnesium oxide appears to be less destructive to vitamins when used as a source of magnesium and, although it has an alkaline pH, it can be buffered to around neutral by dicalcium phosphate (Kohnke, 2002; 2003).

Selenium and chromium from yeast: Sel-Plex® and BioChrome®

These minerals, critical to antioxidant protection (Se) and energy utilisation (Cr) are more easily metabolised and retained than inorganic forms (Dunnett, 2003).

Mineral proteinates: Bioplex® trace minerals

Bioplexes supply key trace minerals in forms similar to those in plants: chelated to amino acids and peptides. In addition to being more available to the animal, these ‘organic’ mineral forms do not trigger oxidation and destruction of the fat-soluble vitamins in premixes. A study to compare the digestibility of inorganic and Bioplexed™ forms of copper and zinc, two very important trace-minerals required for cartilage formation in growing horses, indicated there was a significant advantage from the inclusion of Bioplex™ forms in the diets. These included higher copper digestibility, daily retention, retention as a percent of intake, and significantly higher apparent daily zinc retention, compared to those supplemented with inorganic forms of copper sulfate and zinc oxide (Baker, 2002).

Copper, zinc and manganese, along with selenium and vitamin E, are important factors in natural antioxidant defence. It is important to include adequate levels of antioxidant nutrients to limit the rate of lipid peroxidation and rancidity, particularly in feeds fortified with omega-3 (n-3) and omega-6 (n-6) fatty acids from vegetable oils. Omega-3 fatty acids are particularly prone to oxidation during storage, and additional levels of antioxidants help protect omega-3 fatty within muscle cell membranes once the feed or premix is consumed (Dunnet, 2003).

Careful formulation to reduce incompatibilities, chemical reactions, binding and known nutrient interactions will not only improve bioavailability of minerals and trace minerals, but minimise the need for excessive overages of vitamins and extend the shelf-life of premixes and supplements.

Conclusion

The soundness of the skeletal structure in growing and exercising horses is largely dependent on providing an adequate diet with a balanced intake of bone and joint structural nutrients during the formative years of a horse’s life. Controlled exercise will assist in the skeletal development and allow remodeling in response to loading in both growing and exercising horses.

Care when formulating feeds, premixes and supplements to ensure optimum bioavailability and stability of skeletal nutrients is essential to maintain long term athletic soundness in all classes of horses.

References


Of caterpillars and horses: recent scientific progress on the cause of Mare Reproductive Loss Syndrome

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Introduction

An abortigenic disease, now known as Mare Reproductive Loss Syndrome (MRLS), significantly impacted the horse industry in the central Ohio Valley in late April and early May, 2001 and 2002. In 2001, approximately 25% of all pregnant mares aborted within several weeks (over 3,000 mares lost pregnancies), and abortion rates exceeded 60% on some farms. MRLS struck hard and without warning, was caused by something in the environment, was not transmittable between animals, and was not associated with any known abortigenic agent or disease. It is a newly recognized disease, and it cost the state of Kentucky ~$330 million in 2001 alone (Thalheimer and Lawrence, 2001). Initial research in the summer and fall of 2001 was targeted toward specific agents, such as mycotoxins (Newman, 2003), fescue toxicosis (Schultz and Bush, 2003), cyanide (Harkins et al., 2003), mandelonitrile (Camargo et al., 2002), weather (Priddy et al., 2003), ionic imbalance and poison hemlock (Powell, 2002), among others. To date, none of these factors has been demonstrated to reproduce MRLS. A comprehensive review of the history of MRLS and the initial experiments is provided in Powell et al., 2003.

An epidemiologic survey conducted by Drs. Roberta Dwyer, David Powell and co-workers revealed a temporal correlation between MRLS and presence of eastern tent caterpillars (ETC; Malacosoma americanum) on horse farms (Dwyer et al., 2003). A statistical correlation does not necessarily mean that the caterpillars caused the abortions, so several groups of scientists from around the country designed experiments to determine the role ETC play in MRLS.

The experiments summarized here are the results of collaborative efforts by Dr. Karen McDowell (Veterinary Science, University of Kentucky), Dr. Bruce Webb (Entomology, University of Kentucky), Dr. Neil Williams and Dr. Mike Donahue (Livestock Disease Diagnostic Center, University of Kentucky), and Dr. Kyle Newman (Venture Laboratories, Lexington, Kentucky). The studies have followed a deductive approach, with the results of each experiment defining the questions posed in the next experiment. Details of the first four experiments can be found in Webb et al. (2004). For all of these experiments, pregnant mares were housed communally on grass paddocks and supplemented with hay as needed, with water available ad libitum. Pregnancies were monitored by manual palpation and real-time ultrasonography weekly during the first experiment, and daily in all subsequent experiments, beginning prior to the first day of treatment and continuing for at least 30 days. Aborted fetuses and placentas, as well as blood samples from the mares, were taken to the Livestock Disease Diagnostic Center (LDDC) for necropsy, histopathology, bacteriology, serology, and virology.

Eastern tent caterpillars cause mare abortions

In April and May, 2002 (the first opportunity to perform experiments with ETC after the 2001 MRLS outbreak), two experiments were conducted to determine if ETC could actually cause fetal loss in pregnant mares. For 20 days (first experiment) or for 10 days (second experiment), for 6 hrs each day, pregnant mares were placed on grass pasture in 16 x 16 ft pens, and ETC were released into the grass in the pens. Each day all pens were moved to a new area of pasture so that the mares would have fresh pasture to graze while in the pens.
In the first experiment, 18 of 29 mares exposed to ETC and/or nest materials lost their pregnancies. In the second experiment, three of seven mares exposed to ETC lost their pregnancies (P<0.05), while none of six mares exposed to ETC frass (excrement) and none of the seven control mares (not exposed to ETC, ETC frass or nest materials) aborted. Signs of abortion were consistent with MRLS, e.g. few to no premonitory signs of impending abortion in the mares, increased echogenicity of fetal fluids prior to fetal death and abortion, and presence of actinobacilli or non-beta-hemolytic streptococci in fetal fluids and fetal tissues. In the second experiment, blood samples were collected from the mares daily for hematology, and every third day for clinical chemistry and serum progesterone and estradiol concentrations. None of these measurements were good predictors of which mares were affected by MRLS. Serum concentrations of both hormones declined associated with abortion, but the decline was after changes in fetal fluid echogenicity were apparent. Results of the first experiment were released to the public on April 26, 2002, before MRLS abortions were reported in the area, and in sufficient time for farms to take steps to reduce exposure of pregnant mares to ETC.

Effects of eliminating pasture grass, and freezing or autoclaving ETC

In the next experiment (August 2002), the effect of pasture was removed by mixing ETC treatments with sweetfeed, and feeding the mares from feed buckets. Additionally, the effects on abortigenic activity of freezing larvae or exposing them to high heat by autoclaving were tested. Finally, whether another hairy caterpillar, gypsy moth caterpillar (GMC, Lymantria dispar), could cause abortions in pregnant mares was evaluated. Pregnant mares, ~40-120 days gestation, were fed ETC larvae that had been collected during April and May, 2002 and stored frozen at -80°C. Pregnant mares (~45-100 days gestation) were fed individually from feed buckets, with treatments (representing 50 g ETC larvae/mare/day for 10 days) mixed in sweetfeed. Three mares fed frozen ETC aborted (P<0.05) with signs consistent with MRLS, and none of the mares fed autoclaved ETC aborted. One mare fed frozen GMC aborted. Signs of abortion in the ETC-fed mares were consistent with MRLS, but the abortion in the GMC-fed mare was not. The fetus from the GMC-fed mare did not grow at a normal rate and there was not an increase in fetal fluid echogenicity prior to abortion, as is typical of MRLS. This experiment demonstrated that the abortigenic activity of ETC was stable to freezing and was destroyed by autoclaving. Whether the abortion in the mare fed GMC was due to treatment or would have occurred regardless of treatment cannot be determined from this experiment.

The abortigenic activity is present in the caterpillar exoskeleton, and cannot be extracted with aqueous or organic solutions

Two experiments, performed in May and June, 2003, were designed to determine which physical portion of the insect was abortigenic, and whether the activity could be extracted with aqueous or organic solutions. ETC larvae were collected in central Kentucky in April and May of 2003 and stored frozen at -80°C. Pregnant mares (~45-100 days gestation) were fed individually from feed buckets, with treatments (representing 50 g ETC larvae/mare/day for 10 days) mixed in sweetfeed. In the first experiment, 35 mares were randomly divided into seven treatment groups of five mares each. For the first two treatments, mares were fed ETC and served as positive controls, or fed saline and served as negative controls. For the next three treatments, mares were fed ETC that had been carefully dissected into three portions, the exoskeleton, including setae (caterpillar hairs), the gut, or the remainder of the internal insect tissues. Each of the three portions of the insect constituted a separate treatment fed to mares. For the final two treatments, ETC were homogenized in saline and then separated by size (larger than 0.45 microns or smaller than 0.45 microns), and mares were fed either the larger sized fraction or the smaller sized fraction. Fetal losses occurred in all five mares fed ETC, in three mares fed ETC exoskeleton (P<0.05), and in one mare fed the larger sized fraction of the homogenized insects. No losses occurred in the negative control (saline) group, in mares fed other ETC tissues (gut or internal tissues), or in mares fed the smaller sized fraction of the homogenized insects. Increased echogenicity of fetal fluids prior to fetal death and bacteriologic findings in fetal tissues (non-beta-hemolytic streptococci or actinobacilli) were consistent with MRLS as the syndrome is recognized in the field. This experiment demonstrated that the abortigenic activity was associated with the
The exoskeleton of the insect and that it could not be extracted with an aqueous solution.

The second experiment was designed to further fractionate the ETC exoskeleton, in order to learn more about the physical and/or chemical nature of the abortigenic activity. Twenty-five pregnant mares were randomly divided into five treatment groups of five mares each. For the first three treatments, mares were fed dissected ETC exoskeleton, (positive controls), corn oil (negative controls), or ETC exoskeleton that had been crushed with a mortar and pestle to disrupt its physical integrity. For the remaining two treatments, lipids were extracted from the ETC exoskeleton with an organic solvent, methylene chloride. The methylene chloride was then evaporated through corn oil, transferring the extracted lipids to the corn oil. The two treatments consisted of the corn oil containing the extracted lipids or the exoskeleton after it had gone through the organic extraction process.

Fetal loss occurred in three mares fed ETC exoskeleton (positive control) (P<0.05), in one mare fed powdered exoskeleton, and in two mares fed exoskeleton after the organic extraction. Again, increased echogenicity of fetal fluids prior to fetal death and presence of non-beta-hemolytic streptococci or actinobacilli in fetal tissues and fluids indicated that the losses were due to MRLS.

Taken together, these two experiments demonstrate that the abortigenic activity of ETC is associated only with the exoskeleton of the caterpillar, it is reduced when the physical integrity of the exoskeleton is disrupted, and it is not easily extracted with either aqueous or organic solutions.

ETC larvae were collected in central Kentucky in April and May of 2003 and stored frozen at -80°C. Ten pregnant gilts, at 54-56 days of gestation at the beginning of the project, were randomly assigned to receive ETC (40 g ETC larvae/gilt/day for 10 days, mixed with their normal gestational ration; n=5 gilts) or to serve as controls (normal gestational ration only; n=5 gilts). Gilts were then randomly paired, with one ETC-fed gilt and one control gilt in each pair.

Two of five gilts fed ETC aborted their entire litters (P=0.4444). Those two gilts and their paired controls were euthanized the day following fetal loss. The remaining three ETC-fed gilts and their control pairs were still pregnant at the time of euthanasia, 29 days after the first ETC treatment. Of particular interest was the identification of caterpillar setae (hairs) embedded in the alimentary tract of all gilts fed ETC but in none of the control gilts (P=0.0079). The setae were surrounded by eosinophilic microgranulomatous lesions and were randomly scattered throughout the alimentary tract of each gilt. Caterpillar setae were not found in other gilt tissues examined, and no other significant macroscopic or microscopic changes or disease processes were identified in the gilts or the fetuses.

This study demonstrated that domestic pigs are a good model for the study of MRLS. It also provided the first indication that ETC can cause fetal death and abortion in a non-equid species, and provided the first indication that ETC cause physical and potentially harmful lesions in the digestive tract of mammals. We have since confirmed that ETC cause similar lesions in the alimentary tract of mares.

Where do we go from here?

The evidence is overwhelming that ETC cause MRLS. At present, the only known way to prevent the disease is to avoid contact between pregnant mares and ETC. On some farms that is an adequate solution; on others it is not. There are many unanswered questions, from both practical and scientific perspectives. Some of these are:

- What is the minimum level of exposure necessary to cause abortions (how many caterpillars does it take and for how long)? When several mares are exposed to the same levels of ETC, why do some abort while some do not (what makes one mare more/less susceptible than the next)? What role does the mare’s immune system play? Does the abortigenic factor/agent persist in the environment after the several
weeks in the spring when ETC are present? Can other
caterpillars/insects cause the disease? Can mares be
treated before exposure to prevent the disease, by
immunization, by feed supplements, or by some other
means? Can mares be effectively treated after
exposure? The answers to these questions, and the
new knowledge gained in the process of asking, will
certainly further our understanding and management
of MRLS. Such studies are also likely to provide
new insights into the of other fetal/placental diseases,
and into the physiology of normal pregnancy in horses.

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The pre-and postharvest application potential for Crop-Set™ and ISR 2000™ on citrus

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**Introduction**

Crop-Set™ and ISR 2000™ are products intended to enhance growth, yield responses and disease resistance in a variety of crops, including citrus. Responses such as fruit size and number increases, and internal quality improvement have been obtained by applying the products to a number of cultivars, including Valencias, Navels, mandarins and grapefruit, at various sites in the world. However, from a commercial perspective, considerable confusion exists as to the most desirable timing of applications for intended plant responses. In addition, the appropriate usage of the products may not always be understood. These issues are vital for the successful usage of the products.

An understanding of the growth pattern (phenological cycle) has been useful in a number of tree crops, and most notably in avocado (Whiley *et al.*, 1988) for making management decisions. If the growth pattern of the tree is taken into account, and linked with the known constituents or perceived activity of applied compounds, then the optimal timing for desired effects can be determined.

The objectives of this paper are to outline the potential for applying the products to attain particular desired effects through using a generic phenological cycle for citrus tree growth and fruit development during a cropping season, by understanding the physiological state of the trees and matching the product applications to this. The theoretical timing and application of the products will be confirmed with known effects of Crop-Set™ and ISR2000™, as demonstrated by experimental evidence, where this is possible.

**The phenological cycle**

Little has been done to outline the phenological cycle of citrus growth within any major production area. However, sufficient generic information relating to the order of growth for various plant parts is available, and can thus be used. With the exception of the fruit maturity rate, which can vary considerably with cultivar and environmental conditions, the cycles of vegetative growth, root growth and flowering are remarkably similar in most subtropical or Mediterranean areas of the world. There is almost no growth cycling in true tropical areas (Davies and Albrigo, 1994), and thus these regions will not be considered. Therefore, a generic phenological model can be used as a basis for management, suitably adapted in terms of time scale, for any production area. Such a generic phenological cycle, based on northern hemisphere dates, is shown in Figure 1.

A shoot flush is usually initiated in spring, when the temperature rises above 12.5°C (Davies and Albrigo, 1994) or after alleviation of water stress (Mendel, 1969). This is followed by a summer flush approximately three months later. Little substantial vegetative growth occurs during the rest of the year, particularly in subtropical or Mediterranean areas. The spring flush (March – April in the northern hemisphere) is usually far more intense, affecting more growing points than the summer flush (January – February).

Root flushes normally occur during the periods between vegetative growth flushes. Bevington and Castle (1985) found the major root flushes to occur from May through June and August through September (the latter being the major flush) in Florida. Stimulation of root growth is both temperature and water dependent, with a minimum temperature of 7°C considered a threshold, although rootstock may also play a role. Perhaps of more
The pre- and postharvest application potential for Crop-Set™ and ISR 2000™ on citrus

importance, however, is the physiological effect of root activity. Wilcox and Davies (1981) reported considerable increases in hydraulic conductivity as root temperature increased from 10 to 30°C. Nutrient uptake in particular is affected. Thus in late winter or early spring if root temperature is still low, yellowing (chlorosis) may occur, which will undoubtedly affect photosynthetic capacity of the tree and thus fruit set, growth and retention. In addition, plant growth regulators are affected, with less movement of cytokinins from the roots to shoots and thus less vegetative bud break. The consequences will be further discussed in relation to flowering. Poor root activity and thus water uptake and movement, may also increase the levels of the plant growth regulator abscisic acid (ABA) with resultant stomatal closure (Zeevaart, 1999), and consequent decreased photosynthetic activity, and altered nitrogen metabolism (King and O'Donoghue, 1995), which may change flowering patterns (Lovatt et al., 1998) through elevated ammonia levels. Late winter or early spring, just prior to bud break, therefore presents an opportunity to intervene and thus modify the roles of available endogenous tree nutrition factors and plant growth regulators such as cytokinins, thus antagonising the effects of ABA.

Citrus flowering has been extensively reviewed (Davenport, 1990; Krajewski and Rabe, 1995). Flower bud induction commences during the winter rest period due to cold or dry conditions. The degree of induction is proportional to intensity of the inductive conditions (Southwick and Davenport, 1986). During this period, reversal to a vegetative state is possible should gibberellic acid be applied (Davenport, 1990). However, once differentiation occurs, the buds will not revert (Lord and Eckard, 1987). The rate of bud development depends on climate (degree days) and inflorescence type (Lovatt et al., 1984), but it can be assumed that macroscopic differentiation occurs during December – January, and visible buds in February (northern hemisphere). Anthesis then occurs in April.

An important characteristic of flowering shoots in citrus is that the inflorescence can range from floral only, to predominantly leafy, with mainly new flush leaves and few flowers. The more leafy inflorescences result in greater fruit set or retention (Monselise, 1986). This could be due to the carbohydrate contribution of these leaves to early fruit growth, or better vascular development due to plant growth regulators produced by the leaves (Erner and Bravdo, 1983). This in turn could enhance the movement of carbohydrates, water and plant growth regulators, which are of particular importance (Erner et al., 2000) to the fruit sink strength and thus growth. Stress periods, when gibberellic acid levels are low or when auxins decrease in the fruit as cell division slows (Cleland, 1999) would be of note. Under these conditions, ABA may predominate, resulting in fruit abscission. The degree of leafiness appears related to the stress (water or temperature) intensity of the induction phase (Lovatt et al., 1998). Manipulation of leafiness characteristics is clearly an opportunity for increasing fruit set.

Once anthesis has taken place, fruit growth occurs. Citrus fruit has a sigmoidal growth pattern. The first phase is one of cell division, which takes approximately six weeks, and is characterised by two
drop periods, one from flowering for three to four weeks, followed by a second (June drop in northern hemisphere) as cell division begins decreasing. This is probably related to plant growth regulator changes. Gibberellins may play a vital role in fruit set and early growth (Cleland, 1999) and are particularly important in decreasing fruit drop (Talon et al., 2000) while cytokinins promote cell division and with auxins enhance vascular development. This would link with the effects of inflorescence leafiness.

After the cessation of cell division, fruit cell differentiation takes place, followed by a period of cell elongation (phase 2 growth). Application of auxins at the end of the cell division phase enhances pulp growth (Agusti, 2000) resulting in larger fruit. This is due to expansion of the juice vesicles (Agusti et al., 1995), which relies on maintenance of turgor, which in turn requires inflow of sugars to the fruit. The cell expansion phase is indeed the period when loading of sugars occurs, mainly from adjacent leaves (Koch and Avigne, 1984). Agusti (2000) states that application of auxins at the start of this phase enhances sucrose deposition in the fruit, which not only increases sugar content, but also ensures turgor and larger fruit size. The cell enlargement phase varies with cultivar and climate, but is typically three to four months (Davies and Albrigo, 1994). Thereafter fruit maturation occurs.

Fruit maturation is characterized by a decrease in juice acid, small increase in sugars and change in rind colour. The time taken for this process varies considerably with climate and cultivar, with temperature being the predominant factor. Plant growth regulators play a role. Application of gibberellins decreases the rate of chlorophyll loss (Agusti, 2000), while other unpublished results appear to indicate that auxin applications at physiological drop enhance colour development. Auxin applications near harvest can delay fruit senescence and prolong hanging of fruit, as is done in some countries. As rind maturation progresses, so susceptibility to postharvest pathogens increases (Angioni et al., 1998). Application of gibberellins or cytokinins (anti-senescence) may slow this process.

Opportunities for Crop-Set™ usage

Crop-Set™ is a natural product derived from an extract primarily of the yucca plant. As such, natural plant growth regulator activity such as expected from cytokinins, gibberellins and auxins are present. In addition, the product has been fortified with micronutrients.

Considering the growth pattern of citrus as outlined, the first window of opportunity would be after flower induction has already occurred, but before visible buds are present. This could be in approximately December – January in the northern hemisphere. The objective at this stage would be to increase the leafiness of the spring growth flush, and thereby improve the potential for fruit set (retention) and growth. The cytokinin-like activity in the product could enhance the potential for bud break, as well as cell division, while gibberellins would enhance leafiness and decrease flowering (Guardiola et al., 1982). The nitrogen and micronutrient content of the product could also be beneficial for fruit set, if applied at this stage. Care needs to be taken that application is not too early, as bud reversal from flower to vegetative could occur (Davenport, 1990). The appropriateness of the application will also depend on season and cultivar. The product should be used where it is expected that heavy flowering will take place, so as to enhance the leafiness of the blossom (El-Otmani, 2000) and as a consequence improve fruit set or retention.

The second window of opportunity is logically at flowering. Work done by Medina (2003) on navel oranges in Brazil showed that an application of Crop-Set™ at the end of blossoming (approximately the end of cell division) resulted in larger fruit size without any decrease in fruit number. Navels are not sensitive to gibberelin applications at fruit set (Agusti et al., 1982) although many other cultivars are. Cytokinins applied to fruit shortly after set (Guardiola, 2000) enhanced fruit retention. Thus, an application of Crop-Set™ at 100% petal fall could be advantageous in retaining fruitlets.

For similar reasons, the third opportunity for Crop-Set™ application is at the end of cell division. At this stage, intense competition for available carbohydrates occurs, and application of compounds with cytokinin-like activity may aid carbohydrate flow to the fruits (Kreidemann, 1968). The effects of auxins are clear, as previously discussed, and the role of micronutrients such as zinc and boron should not be ignored. This is also a critical period in terms of adverse effects of environmental stress. A product such as Crop-Set™ could mitigate the effects of ethylene production during stress periods, thus improving the chances of fruit set and growth. The issue of carbohydrate movement toward fruits is also important, considering that the next phase of fruit
growth (cell enlargement) coincides with the start of sugar accumulation, which therefore relates to final fruit brix content. Work done by Bower (unpublished data) showed that applications of Crop-Set™ on Valencia oranges at six weeks post-petal fall (end of cell division) or at six weeks plus at 11 weeks post-petal fall, substantially enhanced final fruit number. Application also changed size distribution to a larger size grouping, as indicated by the number and percentage of total fruits in the most economical size range of 40 to 88 (15 kg carton). Results are shown in Table 1.

Similar research was conducted in South Africa on grapefruit, where fruit size is a considerable problem, enhanced by the presence of citrus tristeza. Results are shown in Table 2 for the Star Ruby cultivar, which is particularly susceptible to production of small fruit. Further, similar results from South Africa and Florida have been published by Marais and Frank (2003).

Opportunities for Crop-Set™ usage later in the fruit growth cycle are questionable. A third spray in the South African Valencia trial at 16 weeks post-petal fall had no positive effects, and in fact resulted in a poorer fruit count distribution than the control. The reasons are unclear, but in considering the phenological cycle (Figure 1), application would have coincided with the summer growth flush. The product could, as previously explained, have stimulated this flush (as it would have done to the leafy blossom). If this were correct, then carbohydrates would have been diverted to the leaf flush rather than the fruit, resulting in poorer size and internal quality. Thus, it is considered that a mid-summer application of Crop-Set™ should not be considered.

Opportunities for ISR 2000™ usage

ISR 2000™ is a product containing Crop-Set™ plus a yeast cell wall component containing a mannan oligosaccharide. The latter is believed to create an induced resistance response in plants. Bower (2003) showed that the product, when applied to navel orange trees three weeks prior to harvest, did indeed induce a response that inhibited the growth of the postharvest pathogen Penicillium digitatum. While the inhibition was not sufficient to be commercially acceptable on its own, efficacy was statistically indistinguishable from that of Imazalil fungicide treatment when combined with a postharvest yeast application (Table 3).

Considerable potential exists for the use of the product in eliminating use of traditional fungicides, to which consumers are becoming increasingly averse (Lopez-Garcia et al., 2000), and solves the problem of poor competitive inhibition of the pathogens by most of the biological products presently available for postharvest pathogen control. In addition, the

Table 1. Effect of Crop-Set™ applied at 6 weeks post-petal fall (PPF) and 6 plus 11 weeks PPF on fruit number, economic size range and total soluble solids at harvest for Valencia sweet oranges in South Africa1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean per tree fruit number</th>
<th>Mean per tree fruit number counts 40-88</th>
<th>Fruit counts 40-88 (%)</th>
<th>Total soluble solids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>212.6 a</td>
<td>168 a</td>
<td>79</td>
<td>9.6 a</td>
</tr>
<tr>
<td>1 spray: 6 weeks PPF</td>
<td>365.9 b</td>
<td>282 b</td>
<td>77</td>
<td>10.3 b</td>
</tr>
<tr>
<td>2 sprays: 6 weeks plus 11 weeks PPF</td>
<td>353.7 b</td>
<td>261 b</td>
<td>74</td>
<td>10.1 b</td>
</tr>
</tbody>
</table>

1Applied at 20 ml product per 100 l water
2Fruit counts per 15 kg carton
abSignificance is indicated at P=0.05 by different letters within a column

Table 2. Effect of Crop-Set™ applied 4 and 8 weeks post petal fall (PPF) on fruit number and economic size range for Star Ruby grapefruit in South Africa1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean per tree fruit number</th>
<th>Mean per tree fruit number counts 32-56</th>
<th>Fruit counts 32-56 (%)</th>
<th>Number of cartons (15 kg) counts 32-56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>243.2 a</td>
<td>169.5 a</td>
<td>69.7</td>
<td>3.9 a</td>
</tr>
<tr>
<td>Crop-Set™</td>
<td>320.5 b</td>
<td>206.0 b</td>
<td>64.2</td>
<td>4.5 b</td>
</tr>
</tbody>
</table>

1Applied at 20 ml product per 100 l water
2Fruit counts per 15 kg carton
abSignificance is indicated at P=0.05 by different letters within a column
Table 3. Effect of pre- and postharvest treatments on growth of *Penicillium digitatum* 21 days after application of ISR2000™

<table>
<thead>
<tr>
<th>Postharvest treatment</th>
<th>Control</th>
<th>Imazalil</th>
<th>ISR2000™ (2g/l)</th>
<th>ISR2000™ (4 g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ISR 2000™</td>
<td>2.6&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;ad&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;ad&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Infection score: 1 indicates no growth; 5 indicates complete coverage of the fruit by fungal mycelium.<br/>
<sup>2</sup>Significance (P=0.05) is indicated by different letters.

Product may, by the nature of its constituents, have a positive effect on fruit integrity, by slowing the senescence process. Thus longer shelf life, in addition to enhanced resistance to postharvest decay, can be expected. For these purposes, application should be after colour break, and two to three weeks prior to harvest. The product will, however, require further testing on cultivars other than Navels. Depending upon the rate of fruit maturity, caution needs to be exercised should application time coincide with flower bud induction, as the Crop-Set™ component may decrease blossom. This may, on the other hand, be advantageous if stimulation of a leafy blossom is desired. This is indeed probably the case in Navels, due to a naturally high flower number where there may be benefits to decreasing flower number. Should this not be the case in other cultivars, an alternative product, without the component of Crop-Set™ could be used.

Conclusions

The phenological cycle for citrus can be constructed for any environment, and can therefore be a useful tool for management decisions, which may vary from place to place and indeed from season to season as well as with cultivar and rootstock combination. By following the phenological cycle, growers can target the use of Crop-Set™ and if desired, ISR 2000™, in a manner which matches their needs to manipulate the tree and fruit growth to advantage. Because tree physiology is correctly matched with the constituents of the products, the desired responses, when required, are likely. Should growers follow this scheme, enhanced fruit size and quality can be achieved, without the potential for failure due to inappropriate product usage, as only the physiologically correct applications will be made.

A summary of potential application windows is shown in Table 4.

References


Table 4. Summary of potential application opportunities for Crop-Set™ on citrus taking the phenological cycle into account.

<table>
<thead>
<tr>
<th>Application time</th>
<th>Reason for application</th>
<th>Caution or comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>After flower induction and before visible flower buds (~Dec–Jan in northern hemisphere)</td>
<td>Enhance leafiness of the blossom and improve fruit set and growth</td>
<td>Do not use too early as vegetative reversion may occur. Also avoid if leafy blossom is expected e.g. after heavy crop.</td>
</tr>
<tr>
<td>At flowering (100% petal fall)</td>
<td>Enhance fruit size and decrease fruit drop. May be particularly useful under stressful conditions</td>
<td>May be combined with second application at 6 to 8 wks post-petal fall (just prior to physiological drop).</td>
</tr>
<tr>
<td>At end of cell division (6 to 8 wks post-petal fall) just prior to physiological drop</td>
<td>Enhancement of fruit size and decrease of fruit drop. Enhancement of internal fruit quality</td>
<td>May be combined with a second application 4 wks later.</td>
</tr>
</tbody>
</table>


Wilcox, D.A. and F.S. Davies. 1981. Temperature...
dependent and diurnal root conductivities in two citrus rootstocks. HortScience 16:303-305.
Citrus fruit size and quality: response to Crop-Set™ in North America

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Introduction

Crop-Set™ is categorized as a natural biostimulant by virtue of the fact that it contains a plant extract belonging to a group collectively known as saponins, which have the ability to influence hormonal activity in plants. Crop-Set™ has been used in the citrus industries of the US, Brazil and South Africa for several years. The most extensive use has been in South Africa, where its application commenced almost seven years ago. The product has been used in the citrus industries of Brazil and the US (California, Florida and Arizona) for almost four years.

To be categorized as a biostimulant a compound must be able to influence the hormonal activity of a plant. Not all biostimulants are natural. For example, the following products are also used as plant growth regulators: benzyladenine (Accel) is a highly reactive synthetic cytokinin; trinexapac-ethyl suppresses gibberellin biosynthesis; gibberellic acid (GA3 – ProGibb) and prohexadione calcium (Apogee – a GA biosynthesis inhibitor); propiconazole is a fungicide with growth regulatory properties; and potassium silicate, which also enhances plant growth.

Natural biostimulants such as those derived from saponins, humic acid and seaweed, are more user-friendly and less likely to become restricted by marketing agencies and consumers. The quality control procedure that Crop-Set™ is subjected to makes it unique among other natural biostimulants. This fact results in the effects being consistent and not fluctuating from season to season.

Significant research on the effect of saponins on the growth of plants was conducted by the French plant physiologists Balansard and Pellessier in the 1940s. They made several significant discoveries viz.: (i) saponins reduced the surface tension of tissues and modified the cellular permeability of seed; (ii) low concentrations of saponins stimulated rapid root development, precocious bud formation and increased chlorophyll production and photosynthesis; (iii) seed treatment with saponins affected all stages of growth of plants e.g. seed germination, vegetative, floral and fruiting stages. These researchers concluded that the effect of saponins was analogous to that of true plant hormones, cytokinins, auxins and gibberellic acid (Balansard and Pellessier, 1943; 1944; 1945). Saponins therefore affect plants at the cellular and morphogenetic levels.

Biostimulants not only affect internal plant hormone levels but also promote the production of antioxidants. Research has shown that environmental stresses such as drought, ultraviolet light, herbicide use, high soil salt content and heat damage plants by causing production of free radicals or reactive oxygen molecules. These molecules are strong oxidizing agents and damage lipids, proteins and DNA inside the plant cells. Antioxidants are metabolites and enzymes that scavenge free radicals and thereby protect plant cells from damage (Zhang and Schmidt, 1999).

Studies by plant physiologists in Brazil (Medina, 2003) and South Africa (J. Bower, personal communication) have identified the same reactions observed by Balansard and Pellessier following the application of Crop-Set™ to citrus trees. These include enhanced lateral bud development, stem elongation, enhanced root development, enhanced flower initiation, increased fruit size and weight, increased juice content, enhanced color and reduced heat stress. The effects at the cellular level include increased cell division, increased elasticity of cell walls, re-direction of photo-assimilates to fruit, increased chlorophyll production, reduced absicissic
acid levels, increased uptake and mobilization of nutrients (in particular calcium) and suppression of the effect of viruses on yield and fruit size (Jameson, 2000).

This paper summarizes the results of field trials with Crop-Set™ in citrus orchards located in Arizona, California and Florida during the 2002-2003 season.

Materials and methods

DOSAGE RATE

In all cases the dosage rate was a total of 16 oz per acre. The amount of water used per acre varied from grower to grower, but was never less than 50 gallons per acre when applied using a mist-blower type spray rig.

TIMING OF APPLICATION

Crop-Set™ applications in sweet oranges and mandarins were applied at petal-fall and when fruit was golf ball size. The timing of applications in the lemon trial in Arizona differed from that of the sweet orange and mandarin studies. In one trial Crop-Set™ was applied at a rate of 16 oz per acre at petal-fall and in the second trial the rate was 8 oz per acre at petal-fall followed by 8 oz per acre at walnut size.

EXPERIMENTAL DESIGN AND ASSESSMENT OF FRUIT SIZE

In the majority of the trials the experimental plots were 5-10 acre treated and untreated blocks. Twenty trees were selected at random in each block and the diameters of 20 fruit measured per tree, using a strap-type caliper. The field trials conducted in Clewiston, FL and Yuma, AZ were based on randomized block designs with 6-9 blocks per treatment. Four to 10 trees were selected within each block for determining fruit size, yield and juice quality. In the mandarin trials in California two entire rows (30 trees per row) within 11 acre blocks were treated with Crop-Set™ and 20 trees selected at random within treated and untreated rows in these blocks. Two buffer rows were left between treated and untreated rows. As the mandarin trees were only 3 years old and produced light crops, only 10 fruit per tree were measured to assess fruit size. Statistical analysis was conducted using Duncan’s Multiple Range Test at P = 0.05.

Results and discussion

GRAPEFRUIT

At the trial site in Ft. Pierce, Crop-Set™ increased the yield of Marsh grapefruit in size class 27 to ≥23 by 35.2% (Table 1). The untreated trees produced 35.5% more fruit in the smaller size class 32-40. Crop-Set™ increased the fruit size of Ruby Red grapefruit in the size classes 40-48 and 23-27 by 8% and 5%, respectively. White grapefruit selections generally produce larger fruit than pigmented selections. Certain overseas markets prefer larger grapefruit; and the increase in total percentage of larger fruit in both selections increases the value of the crop significantly.

SWEET ORANGES

Crop-Set™ increased the percentage of Valencia fruit in size class 56-72 at the Ft. Pierce site by 21% (Table 2). Untreated trees produced 21% more fruit in the smaller size category 88-105. At the Frostproof site Crop-Set™ increased the yield of Valencia fruit in size category 48-64 by 22.5% and untreated trees produced 19.5% more smaller fruit in size class 88-105. The increase in fruit size in the Valencia trial at Clewiston was not as dramatic as at the previous two sites but was still fairly substantial viz. 10.2% more fruit in size class 48-72. The untreated trees produced 10% more smaller fruit in size class 88-125.

<table>
<thead>
<tr>
<th>Locality and selection</th>
<th>Treatment</th>
<th>Fruit size categories</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&gt;23 23 27 32 36 40 48 56 64</td>
</tr>
<tr>
<td>Ft. Pierce (Marsh)</td>
<td>Control</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt; 24.5&lt;sup&gt;a&lt;/sup&gt; 34.3&lt;sup&gt;b&lt;/sup&gt; 21.3&lt;sup&gt;a&lt;/sup&gt; 14.5&lt;sup&gt;b&lt;/sup&gt; 4.5&lt;sup&gt;a&lt;/sup&gt; 0.0 0.0 0.0</td>
</tr>
<tr>
<td></td>
<td>Crop-Set™</td>
<td>24.8&lt;sup&gt;b&lt;/sup&gt; 46.0&lt;sup&gt;b&lt;/sup&gt; 24.5&lt;sup&gt;a&lt;/sup&gt; 3.3&lt;sup&gt;a&lt;/sup&gt; 1.5&lt;sup&gt;a&lt;/sup&gt; 0.0&lt;sup&gt;a&lt;/sup&gt; 0.0 0.0 0.0</td>
</tr>
<tr>
<td>La Belle (Ruby Red)</td>
<td>Control</td>
<td>0.0 6.5&lt;sup&gt;a&lt;/sup&gt; 22.0&lt;sup&gt;a&lt;/sup&gt; 19.0&lt;sup&gt;b&lt;/sup&gt; 32.5&lt;sup&gt;a&lt;/sup&gt; 12.0&lt;sup&gt;a&lt;/sup&gt; 3.5&lt;sup&gt;a&lt;/sup&gt; 4.0&lt;sup&gt;a&lt;/sup&gt; 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Crop-Set™</td>
<td>0.0 14.0&lt;sup&gt;a&lt;/sup&gt; 20.0&lt;sup&gt;a&lt;/sup&gt; 18.0&lt;sup&gt;a&lt;/sup&gt; 21.0&lt;sup&gt;a&lt;/sup&gt; 18.0&lt;sup&gt;a&lt;/sup&gt; 5.5&lt;sup&gt;a&lt;/sup&gt; 3.5&lt;sup&gt;a&lt;/sup&gt; 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Means in a column without common superscripts differ (P=0.05).
The increase in fruit size in the Hamlin trial in Clewiston was not as dramatic as in the Valencia trials. Hamlin is a mid-season variety which produces smaller fruit than Valencia. Crop-Set™ treated trees produced 5.8% more fruit in size class 48-72 and the untreated trees 5.9% more fruit in the smaller size class 88-125.

**LEMONS**

Lemon trees can produce from 1-3 crops per season depending on where they are grown. In the desert areas of California only one crop is produced owing to the harsh summer climate. In the coastal areas 3-4 crops can be produced annually. Because of the ‘monsoon-type’ climate in Arizona, lemons trees can also produce more than one crop. Citrus growers with lemon trees that produce multiple crops must increase the fruit size and yield of the crop which is most valuable to them. Timing of Crop-Set™ applications is therefore critical in this citrus variety. This is also to the benefit of the grower as he can manipulate his trees to produce the crop desired to meet market needs and supply the best fruit when his competitors, e.g. Spain and Argentina, are not able to. In this particular trial, the best treatment appeared to be one application of 16 oz. of Crop-Set™ per acre at petal-fall compared with the standard recommendation of one application of 8 oz per acre at petal-fall, followed up by a second application at the same dosage rate (Table 3). Trees that received only one application of Crop-Set™ produced 18% more fruit in total yield than the untreated trees. These trees also produced 17% more fruit in the size class 75-95. The untreated trees produced 18% more fruit in the smaller size class 115-285.

**MANDARINS**

The trees in these trials were only 3 years old and were therefore not expected to respond to treatment to the same extent as mature trees. However, only one of the selections (No. 8) showed no enhanced fruit size, while the remaining three selections all reacted very favorably to Crop-Set™ (Table 4). Not only did these selections show enhanced fruit size, but all four selections showed increased Brix levels. The Brix values for the different selections treated with Crop-Set™ increased by 1%, 1.2%, 2.9% and 4.5%, respectively for selections No. 11, No. 8, No. 16 and No. 10. The fruit size increases for selections No. 10, No. 11 and No. 16 were 14.5% in size class

### Table 2. Effect of Crop-Set™ on fruit size distribution (% of total) of sweet orange varieties in Florida.

<table>
<thead>
<tr>
<th>Locality and selection</th>
<th>Treatment</th>
<th>48</th>
<th>56</th>
<th>64</th>
<th>72</th>
<th>88</th>
<th>105</th>
<th>125</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clewiston (Hamlin)</td>
<td>Control</td>
<td>0.0^a</td>
<td>0.0^a</td>
<td>6.3^b</td>
<td>7.5^c</td>
<td>23.5^c</td>
<td>43.5^c</td>
<td>17.5^c</td>
</tr>
<tr>
<td></td>
<td>Crop-Set™</td>
<td>0.2^a</td>
<td>1.9^a</td>
<td>7.5^c</td>
<td>23.5^c</td>
<td>43.5^c</td>
<td>17.5^c</td>
<td>5.8^c</td>
</tr>
<tr>
<td>Clewiston (Valencia)</td>
<td>Control</td>
<td>0.2^a</td>
<td>4.6^a</td>
<td>18.3^a</td>
<td>46.3^a</td>
<td>26.5^a</td>
<td>3.9^b</td>
<td>0.2^a</td>
</tr>
<tr>
<td></td>
<td>Crop-Set™</td>
<td>1.3^a</td>
<td>10.4^a</td>
<td>25.2^a</td>
<td>42.7^a</td>
<td>19.4^a</td>
<td>0.8^a</td>
<td>0.0^a</td>
</tr>
<tr>
<td>Ft. Pierce (Valencia)</td>
<td>Control</td>
<td>0.0^a</td>
<td>0.0^a</td>
<td>8.0^a</td>
<td>47.5^a</td>
<td>40.0^a</td>
<td>4.3^a</td>
<td>0.0^a</td>
</tr>
<tr>
<td></td>
<td>Crop-Set™</td>
<td>0.0^a</td>
<td>1.8^a</td>
<td>22.5^a</td>
<td>52.5^a</td>
<td>22.8^a</td>
<td>0.5^a</td>
<td>0.0^a</td>
</tr>
<tr>
<td>Frostproof (Valencia)</td>
<td>Control</td>
<td>0.0^a</td>
<td>0.3^a</td>
<td>12.5^a</td>
<td>47.3^a</td>
<td>33.3^a</td>
<td>6.8^a</td>
<td>0.0^a</td>
</tr>
<tr>
<td></td>
<td>Crop-Set™</td>
<td>1.5^a</td>
<td>10.3</td>
<td>23.5^a</td>
<td>44.3^a</td>
<td>20.5^a</td>
<td>0.0^a</td>
<td>0.0^a</td>
</tr>
</tbody>
</table>

^a Means in a column without common superscripts differ (P=0.05).

### Table 3. Effect of Crop-Set™ on fruit size distribution (% of total) of Lisbon lemon in Yuma, Arizona.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fruit categories</th>
<th>Total yield (bins/plot)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>75</td>
<td>95</td>
</tr>
<tr>
<td>Control</td>
<td>16.3^a</td>
<td>46.4^a</td>
</tr>
<tr>
<td>Crop-Set™</td>
<td>13.9^a</td>
<td>41.4^a</td>
</tr>
<tr>
<td>Crop-Set™*</td>
<td>31.4^a</td>
<td>50.6^a</td>
</tr>
</tbody>
</table>

*Denotes one application of Crop-Set™ at 16 oz per acre at petal-fall. ^b Means in a column without common superscripts differ (P=0.05).
Citrus fruit size and quality: response to Crop-Set™ in North America

Table 4. Effect of Crop-Set™ on fruit size distribution (% of total) of different mandarin selections in California.

<table>
<thead>
<tr>
<th>Selection</th>
<th>Treatment</th>
<th>Fruit size categories</th>
<th>Brix</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&gt;60</td>
<td>60</td>
</tr>
<tr>
<td>No. 8</td>
<td>Control</td>
<td>52.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Crop-Set™</td>
<td>52.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. 10</td>
<td>Control</td>
<td>64.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Crop-Set™</td>
<td>79.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. 11</td>
<td>Control</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Crop-Set™</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>No. 16</td>
<td>Control</td>
<td>0.0</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Crop-Set™</td>
<td>0.0</td>
<td>2.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Means in a column without common superscripts differ (P=0.05).

Table 5. Effect of Crop-Set™ on fruit size distribution (% of total) and quality of Minneola tangelo in Yuma, AZ.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fruit size categories</th>
<th>Total yield (lb/tree)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40</td>
<td>48</td>
</tr>
<tr>
<td>Control</td>
<td>10.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crop-Set™</td>
<td>11.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Means in a column without common superscripts differ (P=0.05).

> 60, 21% in size class 81-105, and 62% in size class 60-81, respectively. Untreated trees produced significantly more fruit in the smaller size classes.

MINNEOLA TANGELOS

Minneola tangelo trees in Florida, California and Arizona tend to exhibit alternate bearing characteristics. This means that one year the crop is very light and the next year a large crop may be produced. The active ingredient in Crop-Set™ viz. saponin, has the ability to enhance flower initiation in plants and should therefore be able to eliminate or reduce the alternate bearing characteristic of this variety if applied at the crucial time. The orchard currently under investigation received a third application of Crop-Set™ immediately following harvest to establish whether we could induce the trees to produce as good or even better crops during the 2004 season. The application of Crop-Set™ during the 2003 season increased the overall yield of these trees by 29% and also increased the fruit in size class 40-56 by 58% (Table 5). Crop-Set™ application also increased juice content, solids:acids ratio and decreased peel thickness, though not significantly.

Conclusions

Crop-Set™ been has shown to consistently increase the quality and size of fruit in the citrus varieties discussed above. The timing of applications of Crop-Set™ in lemons appears to differ from that of sweet orange varieties. It is imperative that the potential of Crop-Set™ to initiate flowering be utilized to its fullest potential in varieties that are alternate bearers such as Minneola tangelo and varieties that are shy bearers such as certain mandarin selections. This may mean that either an early fall application to coincide with the last summer flush or an application immediately following harvest be applied.

References


Integrated efficacy of several Improcrop compounds on bacterial wilt of tomato plants under greenhouse conditions

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²Plant Protection Department, Faculty of Agriculture, Ege University, Izmir, Turkey

Introduction

Tomatoes rank second in terms of cash receipts among all vegetables produced in the United States, with a net value of more than $1.6 billion and a total production of 12 million tons (Swiader and Ware, 2002). Bacterial wilt caused by *Ralstonia solanacearum* is a serious disease and a major constraint in production of tomatoes. This disease causes rapid wilt and death of the tomato plant; and incidence can be as high as 50% or more in the southern US and subtropical and tropical areas of the world (Jones *et al.*, 1991).

The efficacy of current strategies for control of bacterial wilt in tomatoes is limited. Conventional pesticides that are known to provide effective control of the disease are not available. As a soil-borne disease with systemic infection through the xylem, bacterial wilt cannot be controlled by foliar application of traditional pesticides such as copper and mancozeb, which are used for control of foliar bacterial diseases. Soil treatments with general-purpose fumigants like methyl bromide did not provide satisfactory control of the disease (Chellemi *et al.*, 1997; Enfinger *et al.*, 1979). Control of bacterial wilt in infested soils is very difficult. It is generally considered that crop rotation with a non-host crop is of minimal value because of the wide range of crop and weed hosts of the pathogen (Hayward, 1991; Pradhanang and Momol, 2001). Once the pathogen becomes established in the field, previously productive tomato fields may have to be abandoned due to serious outbreak of the disease. The destructive nature of the disease and ineffective disease suppression of current control measures have made development of effective disease prevention approaches desirable.

The objective of this study was to evaluate the efficacy of several Improcrop compounds for control of bacterial wilt of tomatoes. The compounds were used for soil and seed treatments in an attempt to reduce pathogen populations combined with application of compounds that may induce resistance of tomato plants for an integrated management of this disease.

Materials and methods

BACTERIAL CULTURE AND INOCULUM PREPARATION

*R. solanacearum* tomato strain Rs5 (race 1, biovar 1) isolated in Quincy, Florida (Pradhanang and Momol, 2001) was used in this study. The bacterial pathogen was grown on casamino acid peptone glucose medium (CPG) (Kelman, 1954) at 28°C for 48 hrs. Bacterial cells were suspended in sterile deionized water and concentration of inoculum was estimated by measuring absorbance at 590 nm. The viable bacterial population was determined following dilution and plating on CPG agar. Soils used for tomato transplanting were infested with the pathogen at an initial density of $6 \times 10^7$ CFU/ml of soil.

APPLICATION OF IMPROCROP COMPOUNDS

Three compounds were used in the study, Stubble-Aid™, ISR 2000™ and Agromos™. Tomato seeds of a susceptible variety (Solar Set) were treated with Stubble-Aid™ solution (50%) for 30 min before
seeding. The seeds were sown in Terra-lite® agricultural mix (Scott Sierra Horticultural Products Co., Marysville, OH) in expanded polystyrene flats with 2.5 cm by 2.5 cm cells and maintained in a greenhouse. The compound ISR 2000™ was applied as a foliar spray and soil drench at a concentration of 1 ml/L starting 15 days before tomato seedling transplanting and once every seven days thereafter for two more applications. Soils (the potting mix) used for transplanting were either treated, or not treated, with Stubble-Aid™ solutions at the rate of 1% two hours after soil infestation with the pathogen. Tomato seedlings were transplanted into plastic ‘Cone-tainers’ (20.5 x 4.0 cm) containing the Stubble-Aid™-treated or not treated soils four days after soil treatment with Stubble-Aid™. Agromos™ was applied as a foliar spray at weekly intervals after transplanting at a concentration of 1.2 ml/L. Nu-Film 17 was used as adjuvant (0.125%) in Agromos™ solution. Untreated tomato plants growing in the infested soils were used as a control. A subset of tomato plants treated (as described above) or not treated with these compounds, was grown in soils not infested with the pathogen to evaluate the effect of these compounds on plant growth. The plants were supplied with Peter’s Peat Lite Special (15:16:17 NPK) at weekly intervals at the rate of 7.5 g /L water.

EXPERIMENT DESIGN AND DISEASE ANALYSIS

A randomized complete block design was employed with five replications and five plants for each treatment in one replication. Disease incidence was recorded weekly after tomato transplanting and quantified as the percentage of plants wilted. Plant height and the weight of roots and foliage were measured at the end of the experiment. The data were analyzed using the ANOVA or GLM procedures of the Statistical Analysis System (SAS Institute, Cary, NC). Means were compared using Duncan’s multiple range test at $P = 0.05$.

Results and discussion

Application of Stubble-Aid™ in conjunction with ISR 2000™ and Agromos™ consistently provided significant protection of tomato plants against bacterial wilt. Ten days after inoculation the protection of tomato plants from $R$. solanacearum was 100% (no treated plants wilted). Twenty-one days after inoculation 24% of inoculated treated plants wilted (Figure 1). Disease incidence of treated plants was 40% four weeks after transplanting while 100% of untreated plants wilted (Table 1, Figures 2 and 3). Application of these compounds also showed a significant enhancement of plant growth as indicated by larger plant and root weight (Table 2).

The results indicated that these compounds exhibited the potential to significantly reduce bacterial wilt incidence and enhance growth of the susceptible tomato cultivar. The mode of action(s) involved in the disease reduction by these compounds is unclear. Treatments of the soil and tomato seeds with Stubble-Aid™ might have reduced populations of $R$. solanacearum in the soil and prevented colonization of the root system by the pathogen. ISR 2000™ and Agromos™ may have the potential to enhance resistance of tomato plants against this pathogen and reduce disease incidence by induced resistance. It is

<table>
<thead>
<tr>
<th>Table 1. Effect of Stubble-Aid™ and other compounds on disease incidence of bacterial wilt of tomato (cv. Solar Set) under greenhouse conditions.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment a,b</td>
</tr>
<tr>
<td>Control (inoculated)</td>
</tr>
<tr>
<td>Control (no inoculation)</td>
</tr>
<tr>
<td>Stubble-Aid + ISR 2000 + Agromos (inoculated)</td>
</tr>
<tr>
<td>Stubble-Aid + ISR 2000 + Agromos (no inoculation)</td>
</tr>
</tbody>
</table>

*aStubble-Aid™ was used to treat seeds and soils, ISR 2000™ was applied as foliar spray and soil drench, and Agromos™ was applied as foliar spray.
*bInoculation or no inoculation indicates seedlings were transplanted into soils infested or not infested with $R$. solanacearum.
*cValues are the means of five replications (25 plants). Same letter in each column indicates no significant difference based on Duncan’s multiple range test at $P = 0.05$.
*dDays after inoculation.
Table 2. Effect of Stubble-Aid™ and other compounds on the growth of tomato plants (cv. Solar Set) in bacterial wilt greenhouse trial.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant weight (g)</th>
<th>Root weight (g)</th>
<th>Foliage weight (g)</th>
<th>Plant height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (inoculated)</td>
<td>0.53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.34&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (no inoculation)</td>
<td>14.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.14&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stubble-Aid + ISR 2000 + Agromos (inoculated)</td>
<td>9.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.79&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.96&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stubble-Aid + ISR 2000 + Agromos (no inoculation)</td>
<td>16.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Stubble-Aid™ was used to treat seeds and soils, ISR 2000™ was applied as foliar spray and soil drench, and Agromos™ was applied as foliar spray (see descriptions in the text for timing and dosage of applications).

<sup>b</sup>Inoculation or no inoculation indicates seedlings were transplanted into soils infested or not infested with <i>R. solanacearum</i>.

<sup>c</sup>Values are the means of five replications (25 plants). Same letter in each column indicates no significant difference based on Duncan’s multiple range test at <i>P</i> = 0.05.

<sup>d</sup>Plant growth was evaluated 36 days after inoculation.

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**Figure 1.** All treatments: photo taken 21 days after inoculation or transplanting.

**Figure 2.** Uninoculated treatments: photo taken at the end of the experiment.

**Figure 3.** Inoculated treatments: photo taken at the end of the experiment.
worthwhile to carry out further experiments to elucidate the mechanisms associated with disease reduction by these compounds. Detailed studies will also be necessary to investigate the timing and application dose needed to sustain the high efficacy that was observed during the early stage (10 days after inoculation). These studies will help optimize the application methodology of these compounds to facilitate their practical use for bacterial wilt management in tomato production.

References


Alternatives against Alternaria: controlling brown spot on Murcott tangors

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²Improcrop Inc., Nicholasville, Kentucky, USA

Introduction

Alternaria brown spot, caused by Alternaria alternata, affects Minneola tangelos, Dancy tangerines, Murcott tangor and less frequently, Orlando tangelos, Novas, Lees and Sunburst tangerines. In rare cases it may also infect grapefruit. This disease causes serious defoliation, fruit drop and fruit blemishes and is a limiting factor in the production of these cultivars in humid areas. Even in semi-arid production areas, blemishes on the peel can significantly reduce marketability of the fruit (Timmer et al., 2000). In humid climates such as that of Florida, fungal diseases such as Alternaria brown spot are difficult to control. Protectant fungicides such as copper products are still the mainstay of control programs for this disease (Timmer, 2002). However, copper accumulates in soils and can cause phytotoxicity (Alva and Graham, 1991); therefore its use needs to be limited in some plantings and in hot, dry weather.

The economics of citrus production preclude frequent application of fungicides. Currently under ideal climatic conditions, growers may apply 9-10 sprays per season to control this disease. These costs are prohibitive and can be as much as $500 per acre (N.A. Smith, personal communication). Strobilurins have recently been registered for citrus and provide good control of Alternaria brown spot (Timmer, 2004). However these fungicides must be managed carefully to avoid future problems with the development of resistance.

Systemic induced resistance has been investigated extensively for the control of many plant diseases. A wide range of compounds such as benzothiadiazoles, salicylic acid, harpin protein, fatty acids and oligosaccharides are known to be effective inducers of plant resistance to disease. Products that induce resistance may be useful in the control of foliar fungal diseases of citrus. It is difficult to control these diseases on rapidly expanding leaves and fruit with protectant fungicides. Induced resistance could therefore provide systemic protection against infection to substitute for, or supplement control by standard fungicides. Products such as Messenger (Harpin protein), ProPhyt (potassium phosphite), Nutriphite (phosphorous acid), Aliette 80 WP (fosetyl-Al), KeyPlex 350DP and 445 DP (α-keto acids), Oxycom Respond (hydrogen peroxide), ReZist, Goemar H11 (laminarin), Serenade (Bacillus subtilis), and Actigard (acibenzolar-s-methyl) have been tested against Alternaria brown spot in the laboratory and greenhouse (Agostini et al., 2003).

In greenhouse studies, most of these products reduced disease severity compared with the untreated control, but were all less effective than the standard Abound fungicide. ProPhyt and Rezist have proven effective when used in early sprays for brown spot control (Bhatia and Timmer, 2003).

The purpose of the current study was to assess the efficacy of Agromos™ and ISR 2000™ to control Alternaria brown spot under field conditions.

Materials and methods

Fungicides were evaluated in an 8-year-old grove of Murcott tangors near Bereah, Florida in 2003. Each treatment was applied to five two-tree plots arranged in a randomized complete block design. Unsprayed guard trees were located between the plots. All sprays were applied with a handgun at 200 psi. The desired rate per acre for mature groves was added to 125 gallons of water and the trees sprayed to runoff using approximately two gallons per tree.
Alternatives against Alternaria: controlling brown spot on Murcott tangors

The first application was made on March 20 when the spring flush was up to 1/4 expanded to reduce inoculum production on old foliage and prevent infection of new growth. A second application was made on April 11 after petal fall to reduce spore production on leaves and protect young fruit from infection. The third application was made on May 13 when fruit was about 1/4-1/2 inch in diameter, approximately 4-5 weeks after petal fall. A fourth application was made on June 19 about 8-10 weeks after petal fall to protect fruit from late Alternaria infection.

Rainfall was very high especially during May and June. Total precipitation between the first and second applications was 6.2 inches, between the second and third it was 8.5 inches and between the third and the fourth it was 20.7 inches. In the period following the last application until July 15, a total of 30.4 inches fell. Thus conditions were highly favorable for disease development this season. However, Murcott is not the most highly susceptible variety and inoculum levels were generally low at the beginning of the season. Despite favorable conditions, damage from brown spot was only moderate on unsprayed controls.

In November, 50 fruit per tree were rated for severity of Alternaria brown spot on a scale of 0 = no disease; 1 = mild disease, suitable for the fresh market; 2-5 = fruit useful only for processing, increasing severity. The percentage of marketable fruit, those rated as 0 or 1, was calculated for each treatment. Data were subjected to analysis of variance and means separated by the Waller Duncan k-ratio t test, P<0.05.

Results and discussion

All treatments reduced disease severity significantly compared to the unsprayed control. Serenade was least effective and all other treatments were significantly more effective than this product.

Some minor phytotoxicity occurred in the treatments where copper fungicides were applied at a rate of 4.3 lb/acre. The phytotoxicity in combination with ISR 2000™ may have been as a result of too high a rate of copper applied to tender leaf tissue. ISR 2000™ contains an ingredient called Crop-Set™, that enhances vegetative growth of citrus (Medina, 2003).

Two applications of either Agromos™ or ISR 2000™ followed by two applications of copper (Kocide 2000) were as effective as four applications of standard copper (Table 1). Four applications of copper resulted in 93% marketable fruit while the combination of copper and Agromos™ or ISR 2000™ resulted in 91% and 92% marketable fruit, respectively. The untreated controls produced 62% marketable fruit.

Agromos™ and ISR 2000™ were not tested alone, but previous results with compounds that induce systemic resistance indicated that they do not achieve the same level of control as standard fungicides (Agostini et al., 2003). This may be true for Agromos™ and ISR 2000™ as well, but these products are more user-friendly than products such as Serenade.

The level of control achieved by these products may not be as high, but organic growers could benefit from them especially if the use of copper fungicides is restricted. Although resistance-inducing compounds such as Agromos™ and ISR 2000™ may not be substitutes for current commercial fungicides, they can be used with great effect when applied earlier to protect rapidly expanding leaf tissue and avoid inoculum build-up. Furthermore, they reduce the number of copper sprays, which in turn reduces the amount of copper reaching the soil.

<table>
<thead>
<tr>
<th>Product</th>
<th>Rate/acre</th>
<th>Applications</th>
<th>Alternaria brown spot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Severity rating (0-5)</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>1.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serenade</td>
<td>4.0 lb</td>
<td>1, 2, 3, 4</td>
<td>0.92&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serenade + Kocide 2000</td>
<td>2.0 lb + 1.0 lb</td>
<td>1, 2, 3, 4</td>
<td>0.35&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serenade + Kocide 2000</td>
<td>4.0 lb + 2.0 lb</td>
<td>1, 2, 3, 4</td>
<td>0.29&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ziram</td>
<td>5.0 lb</td>
<td>1, 2, 3, 4</td>
<td>0.18&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ziram</td>
<td>7.0 lb</td>
<td>1, 2, 3, 4</td>
<td>0.33&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>ISR 2000™ + Kocide 2000</td>
<td>0.63 qt 4.3 lb</td>
<td>1, 2, 3, 4</td>
<td>0.41&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Agromos + Kocide 2000</td>
<td>0.42 qt 4.3 lb</td>
<td>1, 2, 3, 4</td>
<td>0.33&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gem</td>
<td>8.0 oz</td>
<td>1, 2, 3, 4</td>
<td>0.22&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-c</sup>Values in each column with a letter in common are not significantly different.
Conclusions

In conclusion, it can be stated that Agromos™ or ISR 2000™ can be used to control Alternaria brown spot when used in conjunction with a standard fungicide such as copper. It is essential that these products be applied early, during the emergence of the spring flush, to increase resistance and prevent the buildup of inoculum. These products can be used as tank mixes with most pesticides, which means that citrus growers do not need to make additional spray applications to accommodate them. The use of Agromos™ and ISR 2000™ will reduce the number of applications of conventional fungicidal sprays by 50% and may reduce the risk of resistance development when using new generation fungicides such as strobilurins.

References


Seed and soil treatments with a natural fungicide product against some fungal and bacterial diseases of vegetables

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Introduction

Many vegetable seeds are contaminated with fungal and bacterial pathogens prior to sowing. These pathogens usually only become active when suitable conditions occur. The stages of growth in which bacterial and fungal pathogens are most critical are during germination and early seedling growth. Seed and soil treatment is imperative to control seed-borne and soil-borne diseases of vegetables. Many pathogens overwinter in the form of special structures e.g. sclerotia, mycelia and oospores. These pathogens gain access to the seeds through cracks which occur in the testae during germination.

*Rhizoctonia solani* and *Pythium* spp. occur together in most soils and cause damping-off and seedling blight diseases in many plants including pepper, tomato and cotton. A fungicide that will control *R. solani* will not necessarily control *Pythium* as these fungi belong to different taxa. *Pseudomonas syringae* pv. *tomato* is a seed-borne bacterial pathogen of tomato causing specks on leaves and fruit. There is currently no commercially effective seed treatment available to control this disease.

Seed treatment with a mixture of thiram and alumine dust has commonly been used in practice to control seed-borne diseases; however, poor control of damping-off diseases in seedbeds and the field has been reported over recent years, owing to the dominance of *R. solani*, drought stress following planting and poor cultural practices. The use of natural products that exhibit elicitor properties such as Natural Fungicide Product (NFP; Stubble-Aid™), could be useful as an alternative to fungicides to economically control these seed and soil-borne diseases. Under laboratory conditions the minimum inhibition concentrations (MIC) for this product against *R. solani* was 1 µl/ml, and for *Pythium* and *P. syringae* pv. *tomato* the concentration was 2 µl/ml, the efficacy being >97% (Tosun, 2003). The purpose of this investigation was to find a practical solution to minimize the damage caused by the above mentioned pathogens using NFP as a seed and (or) soil treatment.

Materials and methods

The isolates of *R. solani*, *Pythium* spp. *P. syringae* pv. *tomato* were obtained from stock cultures at the Department of Plant Protection, Ege University, Turkey isolated originally from diseased tomato and pepper plant materials. The fungicide standard used was Pomarsol Forte 80 WP (thiram) supplied by Bayer Crop Science and NFP (Stubble-Aid™) was provided by Improcrop USA.

SEED TREATMENTS

For the seed treatments with NFP, two different methods were used: 1) seed was soaked in a 10% NFP + water solution for 30 minutes; and 2) 500 g of tomato and pepper seeds were coated with a special polymer and NFP at concentrations of 60 ml polymer and 40 ml NFP, respectively, using commercial seedcoating equipment. Seed commercially pre-treated with thiram was used as control.

SOIL TREATMENTS

The soil in plastic trays was watered with an NFP
solution (2 L NFP/100 L water) prior to sowing with untreated and treated seed inoculated with bacteria. The trays were treated four times at weekly intervals, using the same solution.

PREPARATION FOR ARTIFICIAL INOCULATION

Fungal inoculum was prepared by inoculating wheat bran in bottles. Once the inoculum had grown sufficiently, artificially inoculated soil media for filling plastic trays was prepared by mixing one part inoculum to 49 parts of peat or soil. One hundred untreated and treated tomato and pepper seeds were planted in the inoculated soil. Each treatment was replicated five times.

For bacteria inoculation, the method was modified from Krüger (1959) and Shoemaker et al. (1976). The surface of the seeds was sterilized with 1:10 sodium hypochlorite and rinsed twice with sterilized distilled water. Bacterial suspensions were prepared at 6 x 10^9 CFU/ml. Four gram aliquots of tomato and pepper seeds were infiltrated with 20 ml bacteria solution by using a vacuum pump for 30 min. Untreated and inoculated seeds were sowed in NFP treated soil. All tests were replicated five times.

EVALUATIONS

The evaluations were carried out after 25 days, when the majority of seedlings in untreated pots were recorded as dead. Re-isolations of fungi and bacteria from the diseased seedlings were also carried out using selective media. In the fungal treatments the ratios of germinated and dead/healthy plants in treated trays were compared with those of untreated trays. For bacteria test evaluations, the numbers of spots on the leaves of plants derived from treated seeds were counted and compare to those of plants from untreated seed.

Results and discussion

Isolates of R. solani and Pythium spp. virulent to tomato and pepper plants and P. syringae pv. tomato isolates from infected tomato were used in the tests. The aforementioned fungal pathogens can cause severe damping off and root rot diseases on many vegetables and ornamental plants resulting in significant yield and economic losses in certain years.

No commercial fungicides are sufficiently effective against them under field conditions. Furrow treatments with special fungicides are not practical or cost effective to growers. Moreover, commercially available seed fungicides are not effective enough to control these pathogens.

P. syringae pv. tomato can cause severe bacterial speck symptoms both in field and greenhouse cultivated tomatoes. Copper compounds only have a residual activity of 7 days and are extensively used. However their accumulation in the soil and the crop are creating great concern in the public sectors and environmental agencies of many countries throughout the world. There is also growing evidence that copper fungicides will not be registered for use in organic farming in the near future.

SEED TREATMENTS

In the tomato experiments the soil surface of most trays was covered with the mycelial growth of R. solani. The results revealed that coating with NFP was not effective against the fungal pathogens under investigation. The mean germination of treated seed was 28% and the ratio of healthy to dead plants was 14:86 (14%). In pepper experiments the mean seed germination was 46% but the survival of plants was an unacceptable 33%.

More promising results were obtained with seed soaked in NFP solution. The mean germination for tomato and pepper seeds was 39% and 54%, respectively. Survival of plants for the two different crops was 31% and 49%, respectively.

Tests with bacterial speck in tomato showed that seed treatment with NFP was very effective against P. syringae pv. tomato. Significantly lower lesion counts were obtained on the leaves of plants derived from seed treated with NFP. The survival of tomato plants was 89%.

In the thiram treatments the percentage germination in pre-treated tomato and pepper seeds was 42 and 55%, respectively. The survival rate of tomato and pepper plants was found to be 38% and 40%, respectively. Thiram was therefore not effective in controlling the diseases caused by the fungal pathogens under investigation.

SOIL TREATMENTS

The most promising results were observed in the tests where NFP was used as a soil drench. No mycelial
growth was observed on the surface of the treated soil. Moreover, this application suppressed the development of *R. solani* and *Pythium* spp. in the soil. The mean germination rate for tomato was found to be 70% and the plant survival rate 68%. The results observed in the pepper trials were more impressive, here the mean seed germination was observed to be 77% and the plant survival rate 81%.

In the bacterial speck trials the results were comparable to those in the above trials. Soil drench treatment resulted in tomatoes having 73% less leaf specks than the plants in untreated soil.

**Conclusions**

In summary, seed treatment with NFP was not effective in controlling the diseases caused by the fungal pathogens *R. solani* and *Pythium* spp., but was effective in controlling bacterial speck caused by *P. syringae pv. tomato*. It is assumed that the bacteria were only on the surface of the seed and not borne internally. If the bacterial pathogen was borne within the seed, the results may have been less impressive. The results obtained in the above study indicate that the use of NFP (Stubble-Aid™) as a soil treatment may be effective on its own or as an integrated measure in controlling soilborne diseases of tomato and pepper, in seedbeds, the greenhouse or field.

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Abiotic stresses and plant activators

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¹Department of Biology, Faculty of Science, Ege University, Izmir, Turkey
²Department of Plant Protection, Faculty of Agriculture, Ege University, Izmir, Turkey

Introduction: abiotic stresses

Since plants are not mobile, they have to endure various abiotic stresses under both natural and agricultural conditions. Among these, drought with its accompanying high temperatures, is gaining more importance due to global warming, which is mainly caused by the greenhouse effect. The likely increase in the evapotranspiration potential caused by an increase in air and soil temperature brings forth other abiotic stresses such as salinity, high temperature and increased ultraviolet (UV-B) radiation. Abiotic stresses are major constraints on worldwide crop production and can account for up to 70% of the yield loss in crop plants. Salinity alone affects productivity on about 80 million hectares of global arable land. Abiotic stresses such as drought, salinity, high temperatures, hypoxia conditions, nutrient deficiency, increased UV-B radiation, herbicides, etc., account for more crop productivity losses than any other factor. Twelve percent of total crop yields are lost due to pathogen infection, which is equivalent to 900 million tons worldwide annually. Some of the abiotic stresses affecting plant growth and metabolism are summarized in this paper.

DROUGHT

Especially in arid and semi-arid regions of the world, water is the major limiting factor for plant growth. As an early response to water deficit, leaf area is decreased and the growth of young leaves is inhibited. Drought stress causes stomata to lose turgor and close to minimize transpiration. Cell division is normally less sensitive, but cell expansion is very sensitive to water deficit. Drought stimulates the production of abscisic acid (ABA) that causes leaves to drop. Growth of shallow roots is inhibited and seedling growth is reduced under water deficient conditions. Water stress usually affects both stomatal conductance and photosynthetic activity as a result of which superoxide radicals are produced and photo-oxidation of chlorophyll (leaf bleaching symptoms) and thereby severe inhibition of photosynthesis occur. Drought also reduces enzyme activity e.g. rubisco, acid invertase, nitrate reductase, nitrite reductase etc. Adaptive responses of plants against drought include cell wall hardening, reduced plant size and growth rate, ABA production and thus arrested growth, stomatal closure and reproduction failure and accumulation of compatible solutes by the cells to provide osmotic adjustment.

SALINITY

While drought and salinity, the major constraints restricting growth and development of higher plants, are quite different environmental conditions, they are frequently discussed together because they cause similar physiological problems for plants. In both stresses, water tends to be lost from plant cells. In addition, salt stress involves both osmotic stress and ionic stress, resulting from high concentrations of potentially toxic salt ions within plant cells. Plants protect themselves from salt stress by excluding toxic ions from the leaves, sequestering them into the vacuoles for maintenance of turgor and synthesizing compounds that aid in keeping water inside the cell, all of which also occur under osmotic stress conditions (Hasegawa et al., 2000). These nontoxic compounds
increase the osmotic potential of the cell and allow normal metabolic processes to continue. Such compounds include mannitol (a sugar alcohol), polyols, sugars, glycinebetaine, sorbitol and proline, which do not intrude upon normal metabolic processes (Hayashi and Murata, 1998) and provide osmotic adjustment by generating a more negative water potential, thereby helping to maintain water movement into the leaf and consequently, leaf turgor.

HIGH TEMPERATURE

Excessive heat caused by high leaf temperature and water deficit can denature enzymes and disrupt metabolism. Evaporation through leaves may lower the temperature of leaves 3-10°C below the ambient temperature. In oleander (Nerium oloaeandar), acclimation to high temperatures is related to a greater degree of saturation of fatty acids in membrane lipids, which makes the membranes less fluid (Raison et al., 1982). Above certain temperature (e.g. 40°C in most temperate plants), plants begin to synthesize large quantities of special proteins called ‘heat shock proteins’. It is suspected that these heat shock proteins like chaperone proteins, help to prevent the denaturing of enzymes by creating a scaffold around the enzyme.

LOW TEMPERATURE

Damage to plant cells occurs when the water in the cell walls and intercellular spaces freezes. Plants respond to cold stress by altering the lipid composition of plasma membranes, e.g. more unsaturated fatty acids are incorporated into the membrane to maintain fluidity. The lower the water potential in these areas the more water leaves the cells, resulting in an increase in the concentration of solutes and lowering the freezing point of the cytosol. Plants in cold regions increase the concentration of sugars in their leaves before winter. Sugars are tolerated in higher concentrations than many ionic salts. Chilling damage can be minimized if plants are first hardened (acclimated) by gradual exposure to cool, but noninjurious, temperatures. During acclimation to cool temperatures the activity of desaturase enzymes increases and the proportion of unsaturated lipids rises (Palta et al., 1993). Desaturation of fatty acids thus provides some protection against damage from chilling.

FLOODING

In flooded soils, the air spaces are filled with water and therefore lack sufficient oxygen to sustain the life of roots. Oxygen deprivation causes the production of ethylene, which causes the cells in the root cortex to undergo apoptosis. This creates air tubes that allow oxygen to reach the flooded roots. Oxygen shortage in roots, like water deficit or high concentrations of salt, can stimulate ABA production and movement of ABA to leaves, resulting in defoliation.

OXIDATIVE STRESS

The role of reactive oxygen species (ROS) such as superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (·OH) and singlet oxygen (^1O$_2$) during biotic and abiotic stress responses has been well documented. In spite of the fact that ROS is detrimental to plants, causing lipid peroxidation, enzyme inactivation, and oxidative damage to DNA, it is a key event causing the induction of systemic acquired resistance (SAR) in the hypersensitive reaction (HR).

Under salt or drought stress, stomatal closure limits CO$_2$ fixation and NADP$^+$ regeneration by the Calvin cycle in the chloroplasts thus enhancing ROS formation. The primary source of H$_2$O$_2$ in the chloroplasts is thought to be the Mehler Reaction (Heldt, 1997):

\[
O_2^- + Fe^{3+}(Cu^{2+}) \rightarrow O_2 + Fe^{2+}(Cu^+) 
\]

In the presence of metal ions such as iron (Fe$^{2+}$, Fe$^{3+}$), superoxide and hydrogen peroxide may react via Haber-Weiss reaction to produce hydroxyl radical, which is highly toxic to biological molecules (Bowler et al., 1992):

\[
H_2O_2 + O_2^- \rightarrow OH^- + ·OH + O_2 
\]

To control the level of ROS and to protect cells against a variety of external stimuli, including biotic and abiotic stresses, plants have a set of defense mechanisms. These mechanisms involve multi-component response systems such as induction of defense genes, induction of systemic acquired resistance, production of pathogenesis related (PR) proteins, accumulation of stress metabolites, enhancement in the activity of enzymes scavenging
ROS (superoxide dismutase, catalase, peroxidases, ascorbate peroxidase, dehydroascorbate reductase, glutathione reductase) and a network of low molecular mass antioxidants (ascorbate, glutathione, phenolic compounds, tocopherols). Reinforcement of cell wall and cuticle are other mechanisms of defense (Breusegem et al., 2001; De Gara et al., 2003). There are many studies showing that under abiotic stress conditions, which induce an overproduction of ROS in cells, plant resistance is achieved by increasing the activity of ROS-detoxifying enzymes or the biosynthesis or regeneration of antioxidant metabolites (Cakmak et al., 1993; Scandalios 1993; Acar et al., 2001; Bor et al., 2003). Therefore, it seems that alteration in the expression/activity of ROS-detoxifying enzymes could also be a key step in the activation of phytopathogen defense.

Reactive oxygen species are thought to be an important element of the HR regulation scheme, together with other secondary messengers like salicylic acid (SA), ethylene, jasmonic acid (JA) and nitric oxide (NO). Some of these events contribute to the limitation of pathogen spread within the infected tissue, while other events, e.g. ion fluxes or changes in protein phosphorylation state, additionally serve as a signal initiating transcription-dependent part of the local response, as well as the starting point for the transduction of systemic signals to distant parts of the plant (Talarczyk and Hennig, 2001). The development of SAR is associated with the expression of a series of genes, including those encoding pathogenesis-related (PR) proteins and phytoalexins, accumulation of ROS, rapid alteration in cell walls and enhanced activity of various defense related enzymes such as peroxidases, chitinases and β(1,3) glucanases.

The plant activators

When pathogens attack plants, they respond by producing signal molecules including SA, systemin, JA and ethylene and the coordinate-expression of a set of genes, many of which encode pathogenesis-related proteins (PR). This acquired resistance (SAR) often results in an enhanced capacity for defense gene activation not only at the site of initial infection, but also in distal non-inoculated tissues, thereby protecting the whole plant from secondary pathogen attack. Plant activators are agents that can effectively ‘switch on’ these plant defense mechanisms before the plant is under attack. Once activated, a plant can naturally protect itself against a broad spectrum of pathogens. In addition, these plant pathways enhance plant vigor and stress tolerance, and increase crop yield and quality by increasing nutrient uptake and photosynthesis within the plant.

NATURALLY INDUCED SAR VERSUS ACTIVATED SAR

In naturally induced SAR the disease continues to develop before SAR becomes fully effective, with the first damage occurring under field conditions. Natural SAR only occurs sporadically and not uniformly across the field. With the application of a Plant Activator, the plant’s defense is activated before the expected onset of disease. The Plant Activator induces SAR in the whole field and not only individual plants, offering a long-lasting protection against a variety of pathogens in different crops.

Conclusion

In the light of reported findings, we hypothesize that plant activators can protect plants from abiotic and(or) biotic stresses by enhancing water retaining capacity by osmotic adjustment, increasing stomatal conductivity, increasing photosynthetic pigment contents, improving photosystem-II efficiency and thus causing an increased photosynthetic rate in plants. Also plant activators might protect many crops from oxidative damage induced by environmental stresses and modulate the regulation of certain defense responses involving the antioxidative system and the local and systemic signal transduction pathways operating under biotic and(or) abiotic stress situations. With the aid of all these effects, plant activators can provide a measure of plant protection leading to higher yields in plants under biotic and(or) abiotic stresses. In the light of this hypothesis, we have initiated an experiment in our Stress Physiology Laboratory, to investigate the effects of plant activators on alleviating the adverse effects of abiotic stress. We hope that this study will contribute to the many aspects of abiotic stress-plant activator relationships.

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Opportunities and dilemmas in molecular aquaculture genetics

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Introduction: transgenic vs enhanced selection

Aquaculture, like agriculture, is beset with problems of cost, price, and risk. Genetic research, in aquaculture as in agriculture, promises – or dangles – solutions to many of these problems “real soon now”. But what kinds of solution? Investors as well as farmers soon notice that there are two, competing styles of modern genetics, which in this paper are loosely termed “transgenic” and “enhanced selection”. These styles are in vigorous competition for public attention and resources. History suggests that the first genetic style to gain a significant lead in solving an aquacultural problem is likely to drive the other style out of the game, probably forever. Inbred/hybrid corn technology effectively stopped the development of open-pollinated, pure-line varieties of corn (Kloppenburg, 1988), and the developers of four-way hybrid poultry have driven pure-line poultry breeding to commercial extinction in developed countries. Commercialization of transgenic salmon which grow twice as fast as existing strains could happen as early as this year, 2004 (Stokstad, 2002; Hoag 2003); if this were to occur classical salmon breeding programs could very well be put out of business. Transgensics with 2X or 4X current commercial growth rates are also available for species of tilapia (Rahman et al., 2001). In tilapia, however, the rate of broodstock improvement through well-conducted selection is so rapid (e.g. Bolivar and Newkirk, 2002) that step-wise transgenic technology may be unable to keep up or even gain a toe-hold.

Both styles of genetics provide useful solutions to aquacultural problems but they have rather different consequences for the industry as well as for the consumer. The winning style in this technology race (more likely than not a winner-take-all race) cannot yet be predicted. This paper describes recent work which is representative of the two styles, in sufficient detail that the current level of sophistication of the science and the size of the outstanding problems can both be appreciated. The topics include possible biological limitations on transgenic growth, progress in transgenic growth and disease resistance, QTL selection, the crucial role of the major histocompatibility complex, and prevention of wasteful reproduction and pointless sexual dimorphisms.

The winner in the commercial (as opposed to technological) race between selection and transgenics will depend to a large extent on the willingness of the public to accept genetically engineered fish. Transgenic fish are looked at with strong disfavour at the present time (Hulata, 2001), but in the long term public opinion is an incalculable quantity. The two styles of genetics also have have different long-term consequences for farmers. Marker-assisted selection and genetic engineering can benefit the owners and developers of the technology and, eventually, the fish-eating public. They will also give transient economic benefits to “early adopter” farmers, but, in the long term, only those farmers who hold exclusive franchises or own part of the technology are likely to benefit from it.

The impossibility of standing still

Most farmers are unwilling simply to ignore genetics, for two reasons. One is the obvious opportunity cost
lost opportunity of forgone genetic improvement. This cost, which can be estimated from simple economic models of the genetic process, is a commonplace of the promotional literature of aquaculture genetics. Opportunity costs calculated from projected farm revenues will, however, be overtaken in the long run by the larger cost of being forever left behind in the technology race. This is a world in which there are promoters, scientists, technology owners, early adopters, follow-on adopters and the fish-eating public. Only the swift-moving early-adopter farmers are likely to gain any long-term competitive advantage from new technology. Large-scale commercial or national genetics programs tend to benefit the public, professional geneticists and the owners of the technology. Economic benefits to individual farmers who are competing with each other in a commodity market are less obvious unless they become early adopters and/or own part of the technology. The situation which confronts farmers is summed up in Alice’s conversation with the Red Queen in the Land of the Looking Glass: “Well, in our country, said Alice, still panting a little, ‘you’d generally get to somewhere else - if you ran very fast for a long time as we’ve been doing.’ ‘A slow sort of country!’ said the Queen. ‘Now, here, you see, it takes all the running you can do, to keep in the same place. If you want to get somewhere else, you must run at least twice as fast as that!’”

Increased growth rate through classical broodstock selection

Mass selection has been by far the most commonly used artificial selection procedure in aquaculture (Hulata, 2001). Hulata’s comprehensive review gives instances of successful mass selection performed on many aquacultural species. Even in common species like Atlantic salmon and tilapia, however, the procedure is sometimes successful (Sanchez et al., 1995) and sometimes not (Teichert-Coddington and Smitherman, 1988; Hulata et al., 1986). The frequent failure of well-conducted mass selection programs in aquaculture has yet to be satisfactorily explained. It is certain, however, that hatcheries that adopt a ‘breed the biggest’ approach to mass selection for growth soon encounter practical problems arising from variation in spawning times and condition of the breeders. The biggest animals may be somewhat older, rather than faster growing, and may then have this initial, non-genetic, size advantage magnified by competition to such an extent that selection for size becomes ineffective.

Straight-forward mass selection often does work, however. In the shrimp Penaeus stylirostris, Goyard et al. (2002) showed that a straight-forward mass selection program can produce useful genetic gains in growth rate. Selection intensities, which ranged between 4% and 18%, were not especially high. The program produced a 21% increase in growth rate by the fifth generation nevertheless. Hetzel et al. (1999) developed two breeding lines in another species of shrimp, P. japonicus, one mass-selected for high weight and one for low weight. The direct response to this selection was an 8.3% gain in weight of the offspring of the high line. The low line lost about 13%. The authors conclude that the realized heritability of growth rate in mass selection is only moderate but the large family sizes and phenotypic variability (opportunity for selection) should permit rapid stock improvement. These remarks apply to all aquacultural fish and shellfish.

Surprisingly, the least successful selection programs tend to have been those which went well beyond simple mass selection in the sophistication of their statistical, quantitative genetic methodologies (i.e. index selection using the so-called ‘animal model’). Several of the most widely promoted projects, continuing for decades in salmon and tilapia, have yet to publish any peer-reviewed proof of genetic gain. Successful index selection has in fact been described in the peer-reviewed literature only twice, to my knowledge (Hershberger et al., 1989; O’Flynn et al., 1999). The realized response to selection in those experiments was not, however, what the authors had predicted from their prior heritability estimates. Selection projects that have worked best in aquaculture have up to now been based on the simpler experimental designs.

It is not at all clear what has gone wrong with sophisticated index selection along classical lines. One possibility is that, as we now know, the genetic architecture of quantitative traits differs from the assumptions made when selection indices are calculated. The genetic basis of the variation in quantitative traits probably involves a few genes having large effects and many genes having small effect (Orr, 1999), contrary to the assumptions of the ‘infinitesimal model’ from which classical heritability estimates are usually derived.
COMPETITION EFFECTS IN SOPHISTICATED SELECTION DESIGNS

A more likely explanation, specific to aquaculture, are the statistical difficulties caused by competition among individual fish within a tank, cage or pond. It has been known for a very long time that competition can have drastic effects both on the estimation of genetic parameters and the outcome of selection programs (Doyle and Talbot, 1986; Jobling, 1983; Moav and Wohlfarth, 1974; Purdom, 1974). Estimates of genetic parameters such as heritabilities and genetic correlations, which are used in sophisticated breeding programs, are distorted because individual error terms in the statistical analyses are not independent, as they are assumed to be in the linear statistical models used to estimate them (Hamblin and Rosielle, 1978; Mazer and Schick, 1991). The food that one animal eats is not available to others; small individuals may be intimidated by others even when food is abundant, etc. The genetic variance component due to the genotypes of other individuals in the population is hidden from ordinary genetic analysis. Wolf (2003) found a negative covariance between direct and indirect (competitive) genetic effects, such that genes that make an individual bigger make other individuals smaller. This effect is surprisingly large, and furthermore it increases as the genetic relatedness of individuals in the competing group increases.

Thirty years ago Moav and Wohlfarth (1974) pointed out that in common carp non-genetic differences in size due to an age difference of only one day become magnified by competition and can dominate the outcome of a selection program. At the present time, however, the lamentable truth is that roughly 99.9% of aquaculture genetic analyses ignore competition despite the fact that it has major non-genetic effects on the variance of growth rate. There are, of course, exceptions, including the work of Moav and his colleagues in Israel. Another recent exception is the work of Brichette et al. (2001) on competitive growth of mussels grown in trays.

The competition problem may disappear in index selection as selection incorporates information from more relatives extending over more generations. Since the competition effect on index and combined selection has not been well studied mathematically, we do not know whether to expect that happy outcome or not. Information derived from DNA markers may perhaps be used to generate the required pedigree structure extending over several generations in populations that are grown and propagated together, as is often the case in aquaculture, but as yet no multi-generation selection experiments based on pedigree markers have been published. The accuracy of pedigree reconstruction from markers is turning out not to be especially high (Thomas et al., 2002; Colman and Slate, 2003; Wilson et al., 2003).

The between-family component of a selection index is always suspect because of non-genetic, environmental effects common to members of a family even when families are reared together in a common tank and DNA markers used to sort them out (e.g., Cunningham et al., 2001; Fishback et al., 2002). The idea that animals in the same tank experience the same environment is an illusion; a family added to the tank on the last spawning day will have a smaller average size than a family added on the first day and will therefore be at a competitive disadvantage. It will also be relatively underfed if feed is supplied to the tank at a rate which is determined by the size and weight-specific metabolic rate averaged across all families.

WITHIN-FAMILY SELECTION

Successful artificial selection programs in aquaculture tend to be those in which the effects of un-analysed competition are minimised operationally (e.g., by selection within families (Uraiwan and Doyle, 1986) rather than statistically. In pure within-family selection, the biggest animals in each (usually full-sib) family are selected as breeders (Hill et al., 1996). Mean differences among families, such as age effects, which can be magnified by competition, are ignored. Because aquacultural species usually produce large broods, selection intensities within families can be high. Bolivar and Newkirk (2002) described a within-family selection experiment in tilapia, which achieved a realized heritability estimate of only 0.14 over 12 generations. However, within-family selection permitted high selection intensities of about 2%. The result was that the size of the fish at 16 weeks more than doubled after 12 generations.

Families must be distinguishable in within-family selection, either by growing them in separate tanks until they are big enough to be tagged or by using DNA markers for identifying families. The latter procedure, called ‘walk-back’ (Doyle and Herbinger, 1994), maximizes effective population size and can achieve very high selection intensities in aquaculture because of the large fecundities. The potential benefit of this procedure over ordinary within-family
selection is that families do not have to be reared separately until they are big enough to be physically tagged.

Selection among families (as opposed to within families) is probably only useful when the selected trait cannot be directly measured on the individual chosen to become a breeder. In practice, this probably limits its utility to selection for resistance to or tolerance of disease when challenge tests are used to identify superior families. Non-challenged and therefore non-infected siblings are used as breeders (e.g. Argue et al., 2002; Henryon et al., 2002; Oliver et al., 2000; Sarder et al., 2001).

**Transgenics and bioengineering**

Much of the current excitement in aquaculture genetics – as in all other areas of genetics – lies in transgenesis and bioengineering. This excitement is fully justified by the rapid progress of the technology for introducing foreign gene constructs into aquacultural species, in the identification of candidate genes and target metabolic pathways for transgenesis, and in the spectacular growth of genetically modified organisms.

The insertion of growth hormone genes has increased the growth of many species of fish (Devlin et al., 2001; Dunham et al., 2001). Fourfold increases without obvious side effects have been reported in salmon (Devlin et al., 1994) and 2.5- to 4-fold increases in tilapia (Rahman et al., 2001). The coding sequences used in transgenesis have sometimes been exogenous, such as human or bovine growth hormone, or have sometimes been isolated from the host species and then spliced to an exogenous, non-transcribed control region to enhance the expression of the gene (Devlin et al., 1995; Martinez et al., 2000).

Aquaculture bioengineering projects with growth hormone have been going on for more than a decade (Fischetti, 1991) and have already resulted in patents such as one granted to A/F Protein Limited (Anonymous, 2000). In the A/FP procedure a salmon growth hormone gene is spliced to a promoter sequence from another fish, the ocean pout, which causes the transgene to transcribe growth hormone in the liver. Unlike the normal salmon gene, which is expressed only some of the time, in the pituitary gland, the transgene is continuously switched on in the liver by its liver-specific promoter. The salmon reach a size of 8 lbs in about 1.5 years. These transformed Atlantic salmon broodstocks are ready for commercial production pending regulatory approval. The review paper by Hulata (2001) discusses the status of growth-rate transgenesis in aquaculture in considerable detail.

An interesting example of genetic engineering which has nothing to do with production is the development of transgenic tilapia with the potential for treating human diabetes (Wright and Pohajdak, 2001). Of course in the ordinary course of things tilapia have evolved to produce tilapia insulin, not human insulin, but genetic engineering has overcome this flaw in the Great Chain of Being. Wright and his colleagues have developed procedures for encapsulating and implanting fish tissue into diabetic mice, where it accurately regulates blood glucose levels. Tilapia and human insulin differ by 17 amino acids but Wright and Pohajdak cloned, sequenced, and modified the tilapia insulin gene by site-directed mutagenesis. The product was a tilapia insulin gene that codes for ‘humanized’ insulin while maintaining all of the tilapia regulatory sequences. They proceeded to develop a strain of transgenic *O. niloticus* that produces humanized insulin along with its normal insulin. Work still needs to be done to replace the normal tilapia gene with the humanized gene by homologous recombination, and/or to make the humanized gene homozygous and adjust the genetic background.

The objective of the project is to use the tilapia as a source of tissue-transplant material for treatment of type II diabetes. Insulin-producing tissue is much easier and cheaper to collect from tilapia than from mammals, so insulin-producing tissue from the genetically modified tilapia should have a markedly lower production cost and, probably, enhanced safety relative to the present mammalian xenogentic transplant donors, which are usually pigs.

**POPULAR PRESSURE TO ACCEPT ‘FRANKENFISH’ (?)**

Public anxiety over the use of genetically modified organisms (GMOs) in food makes it impossible to predict when transgenic aquaculture species will enter commercial production. It is the view of most geneticists that acceptance will be very slow, partly because the public is thought to see nothing beneficial in the technology which would offset the perceived risks. In the case of agriculture this may very well be true, at least in developed countries. The GMOs developed so far have been corn, soybeans etc. modified for the economic advantage of seed
companies, growers and herbicide manufacturers, not the end consumers (Charles, 2001). An editorial in Nature (Anonymous, 1999) sums up this attitude: “GM soybeans? Who needs them?” The same question can fairly be asked about genetically modified aquacultural species, which, with few exceptions, have been developed with increased production in mind.

The dismissal of conventional, production-oriented GMOs may however be expressive of a parochial point of view. The need for GMO technology in some developing countries is actually rather obvious. The answer to the rhetorical question “Who needs them?” may be, “practically everyone in the third world”. Trewavas (1999) makes a case for believing that a new agriculture, combining genetic modification technology with sustainable farming, is our best or only hope for staving off an ecological catastrophe. If we don’t use biotechnology, he says, we are going to run out of arable land and water. The conflict between environmental activists and starving third-world pragmatists made headlines at the World Summit on Sustainable Development, held in August 2002 in Johannesburg. Zambian President Levy Mwanawasa (who had declared a food emergency three months previously) announced that he had stopped the distribution of 17,000,000 kg of corn because some of it is genetically modified. “We would rather starve than eat something toxic”, the President said, voicing the anti-GMO viewpoint of the developed world (Wente, 2002). Not everyone in Zambia agrees. Wente cites a Los Angeles Times reporter who was told by a Zambian, “We don’t care if it is poisonous because we are dying anyway”. Public perception, not science, is the key to the future. Pressure to accept GMO technology which increases yield may bring GMO crops into production in the poorer and more ecologically stressed parts of the world much sooner than many people expect.

BIOLOGICAL LIMITATIONS ON TRANSGENIC GROWTH

Transgenics stands far above all other productivity-enhancing genetic technology in terms of potential payoff and risk. However, there are some preliminary indications that the most direct transgenic procedure, inserting a transgenic growth hormone, may be relatively ineffective in lines which have already been selected for high growth (Devlin et al., 2001; Parks et al., 2000). It appears that selective improvement and transgenesis may not combine additively. To the extent that this turns out to be true in general, selected strains that achieve growth rates comparable to transgenics may remain competitive with transgenics, with much lower development costs.

The effect of a transgene on growth may be strongly influenced by the genetic background of the host. Devlin et al. (2001) compared the effect of a growth hormone transgene in slow-growing, wild rainbow trout with its effect in rainbow trout that had a long prior history of domestication and selection for fast growth. They comment that “the growth response is strongly influenced by the intrinsic growth rate and genetic background of the host strain, and that inserting growth-hormone transgenes into highly domesticated fish does not necessarily result in further growth enhancement.” The growth of the transgenic fish speeded up 17-fold (!) but was still not faster than that of the highly domesticated strain. The domesticated strain hardly responded to the transgene at all. Cranial abnormalities were seen in the transgenic but not in the domesticated animals, which were growing at about the same rate, suggesting that ordinary homeostatic mechanisms were not coping with the novel pathways of growth and development induced by the transgene.

Devlin and his co-authors conclude that “The effect of introducing a growth-hormone gene construct into fish to increase growth rates appears to be dependent on the degree to which earlier enhancement has been achieved by traditional genetic selection. Such effects are likely to be specific for different species, strains and transgenes — in selected mice or in domesticated, rapidly growing farm animals, for example, growth-hormone transgenesis can have little effect on growth or it can induce pathological effects, as we have seen in transgenic salmonids.”

Aquaculture geneticists, like other biologists, may be able to learn a lot from the study of mice. Bunger and Hill (1999) selected lines of mice for high and low body weight for more than 50 generations, after which the high and low lines had diverged approximately 3-fold in their weight at 98 days. The authors then eliminated growth hormone from the metabolism of the mice by genetic ‘knock out’, which they achieved by backcrossing a defective growth hormone (GH) releasing factor receptor gene into both lines. Control high and low lines with the normal GH gene were also maintained.

Both lines of mice carrying the knock-out gene, which were thereby deficient in GH, were much smaller than the normal control mice at 98 days. There is no doubt that growth hormone makes mice
grow quickly. What is surprising is that the divergence of the high and low lines was almost as great in the absence of growth hormone (2.4-fold divergence) as in its presence (3.1-fold). The authors conclude that after appropriate scale transformation, “changes in the GH system contribute only a small part of the selection response in growth ... and other systems contributed most of the selection response”.

This experiment should interest the aquaculture community even though it was performed on mice. We know that transgenic fish carrying extra growth hormone genes, or modified genes that express GH continuously, are fast-growing fish - sometimes very fast-growing. This ingenious knock-out experiment on mice is a hint that the converse may not be true. Selection of fast-growing fish by classical methods may evoke an entirely different kind of genetic change that does not involve growth hormone. Furthermore, it suggests that if crosses between high- and low-selected lines are used in searches for growth QTLs, the growth hormone system will not necessarily provide the best candidate genes.

**TRANSGENIC DISEASE RESISTANCE**

Resistance to disease is of particular interest in the culture of salmonids and shrimp, a fact which is reflected in the focus of ongoing projects in genetic engineering. Standard techniques for inserting foreign genes have been difficult to apply to shrimp because embryos of *Penaeus* are released from their mothers at a relatively advanced stage. Newly-fertilized eggs are essentially unavailable at the appropriate stage for microinjection or electroporation. Sarmasik *et al.* (2001) may have found a way around this problem, which is expected to work in other crustaceans and live-bearing fish. The foreign gene is carried into the host by an extensively engineered viral vector. One engineered feature of the vector makes it unable to replicate. Other features, derived from the hepatitis B virus and the vesicular stomatitis virus (a pathogen similar to hoof and mouth disease which infects mammals, insects and possibly plants), enable the vector to stick to the cell membrane of a wide variety of organisms. Immature gonads of the crayfish were injected with a solution of the vector about one month before the normal age of first reproduction. When they matured the injected individuals were mated with normal individuals. Sarmasik *et al.* (2001) provide proof of integration, expression and transmission of the reporter transgene for at least three generations.

Some of the more promising transgenes for disease resistance are genes encoding lectin molecules. Lectins are small peptides (amino acid sequences) that bind to sugar molecules exposed on the surface of cell membranes. After binding, some types of lectin lyse the phospholipid bilayer of the membrane, killing the cells. Lytic peptides are proving to be potent toxins to a broad range of bacterial, fungal and protozoan pathogens. Much work has gone into producing transgenic plants and mice that express enhanced levels of lectins as built-in fungicides, bactericides and insecticides.

Disease-related transgenic experiments in aquaculture have focused on Cecropin-B, an antimicrobial peptide of about 35 amino acids which is synthesized in the pupae of the silk moth in response to bacterial infection. Electroporation has been used to incorporate cecropin-producing genes into Medaka, with a resulting increase in resistance to *Pseudomonas fluorescens*, *Aeromonas hydrophilia*, and *Vibrio anguillarum* (Sarmasik *et al.*, 2002). Challenge studies showed that while about 40% of the controls were killed by both pathogens, only up to 10% of the F2 transgenic Medaka were killed by *P. fluorescens* and about 10% to 30% by *V. anguillarum*.

A similar transgene construct and insertion procedure greatly increased the resistance of the channel catfish *Ictalurus punctatus* to the epizootic of *Flavobacterium columnare* in an earthen pond (Dunham *et al.*, 2002). Fully 100% of the transgenic catfish survived a natural exposure to the flavobacteria, *versus* 27% survival of normal fish. When challenged in tanks with *Edwardsiella ictaluri*, a bacterium that causes enteric septicaemia in catfish, survival of the transgenic fish was 41% *versus* 15% for the controls.

The use of cecropin transgene in aquaculture has been patented (Cooper and Enright, 1999). The patent claims that “Augmentation of the host’s defences against infectious diseases or tumours is achieved by “arming” the host’s cells with an exogenous gene encoding a natural or synthetic lytic peptide. …The transformed cells have the ability to produce and secrete a broad spectrum chemotherapeutic agent that has a systemic effect on certain pathogens, particularly pathogens that might otherwise evade or overcome host defences.”

**MARKER-ASSISTED AND QTL SELECTION**

The search for disease-resistance and other loci that
have effects which are large enough to be useful but not large enough to be obvious by simple segregation analysis (quantitative trait loci, or QTLs) is following two approaches, marker-assisted selection (MAS) and the search for ‘candidate genes’. Both approaches are expected to be most useful when ordinary quantitative genetic procedures for estimating breeding values have especially low accuracy – in particular, when selecting for disease resistance if the selected animals cannot be exposed to the disease. MAS and QTL selection will be less useful in selecting for growth where heritabilities are usually reasonably high and estimation of the breeding values of individuals from their phenotypes is reasonably accurate. Simulation studies of selection on growth rate, a trait that is measured on all animals prior to selection, find only small gains from addition of marker data (Lande and Thompson, 1990).

A typical example of MAS for a disease-related trait in aquaculture is provided by the work of Ozaki et al. (2001) on QTLs associated with susceptibility to infectious pancreatic necrosis virus (IPNV) in rainbow trout (Oncorhyncus mykiss). Backcrosses between resistant and susceptible strains were used to identify several chromosome regions containing putative QTL genes that affect disease resistance. Fifty-one microsatellite markers were used for the linkage analysis.

Perry et al. (2001) employed a rather similar approach in finding a QTL for upper thermal tolerance in outbred strains of rainbow trout. Segregation at the microsatellite marker for the QTL explained 7.5% of the variance in thermal tolerance in the trout progenies. This is about what we would expect for a relatively large QTL.

The candidate gene approach to finding QTLs involves looking for genetic variants in biochemical or developmental pathways that are known, or strongly suspected, to affect the trait of interest. An example is the work of Schulte et al. (2000) on the lactate dehydrogenase-B gene (Ldh-B) in northern and southern populations of the fish Fundulus heteroclitus. Northern (Newfoundland) fish grow better at lower temperatures while fish from Florida are superior at higher temperatures. The experimental details are too complicated to be easily summarized here but they include temporary transgenesis of the regulatory sequences into the livers of experimental fish; deletion studies to identify the approximate location within the regulatory sequence where the adaptive changes in the transcript occurred; stress tests of live fish to see which alleles (northern or southern) drive the transcription of the gene. A difference of only one base pair in the regulatory sequence accounts for the adaptive difference between the northern and southern populations.

It appears that over the long term, phenotypic selection for quantitative traits may give better results than either MAS or QTL selection. In a simulation study Villanueva et al. (2002) found that selecting for one particular gene allows the other additive ‘background’ genes to drop out of the population by chance (inbreeding rate will usually be an indicator of this effect). Thus there is a loss of additive genetic variance with QTL and MAS selection relative to phenotypic selection, and the ultimate selection limits are lower. Interestingly, the reverse seemed not to happen in the simulation - only very rarely was the advantageous QTL allele lost during phenotypic selection. However, in the short term, both QTL and MAS gave a more rapid initial response than phenotypic selection. Salmon and some other aquacultural species have such long generation intervals that rapid response could actually be worth more, by an economic calculation like net present value, than a high selection plateau that might not be approached for 100 years.

The major histocompatibility complex and disease resistance

Interest in genetic variation in the major histocompatibility complex of fish (MHC) is running high these days. The diversity of the MHC loci, which are the foundation of vertebrate immune systems, appears to be driven by the diversity of pathogens in the environment (Penn et al., 2002). Fish may choose their mates to optimize the MHC genotype of their offspring (Landry et al., 2001). The preferred explanation for this ‘disassortative mating’, in which fish choose mates which are genetically unlike themselves, is that MHC heterozygotes are intrinsically more fit than homozygotes (overdominant selection at MHC loci; Arkush et al., 2002). The thought is that heterozygosity at MHC loci may enhance a host animal’s resistance to pathogens by increasing both the diversity of peptide antigens it presents to T-cells and the diversity of the T-cells themselves.

But there is also evidence (Miller et al., 2001) that particular MHC alleles may be directionally selected, i.e. towards homozygosity, possibly on a lake-specific basis. Heterozygotes would not be more fit than MHC...
heterozygous and disassortative mating should not be selected in such lakes. And in an experiment in an aquaculture-like environment where exposure to specific pathogen strains was controlled, particular MHC alleles appeared to have a selective advantage but heterozygosity did not (Lohm et al., 2002).

Along with laboratory, field and theoretical studies of the advantages of immune-system diversity per se, there have also been demonstrations that specific pathogens can exert strong selection on particular MHC alleles in fish. Lohm et al. (2002) reported on the resistance to furunculosis in Atlantic salmon originating from the Akvaforsk strain currently reared by AquaGen AS in Norway. Families of salmon were mated on the basis of their MHC genotypes so as to control the genetic background against which specific MHC alleles were expressed. Surprisingly, in this controlled breeding experiment MHC heterozygosity per se did not improve resistance to the furunculosis challenge test. It was particular Class II MHC alleles that conferred relative fitness differences as great as 0.5. The authors write, “This study clearly shows a strong survival advantage for individuals carrying a high-resistance allele when exposed to a bacterial infection. ... directional selection acting on the MHC despite its high polymorphism stresses the importance of renewal of genetic variation at these kinds of loci, either from mutation, recombination or immigration from other populations, when combating new or coevolving virulent pathogens.”

A recent study by Cohen (2002), which delved deeper into the molecular structure of the MHC antigen-binding sites, could be a harbinger of more powerful ways to investigate and exploit MHC variation in fish. A population of Fundulus heteroclitus was found to have adapted to an environment which has been grossly polluted with PCBs and other contaminants for more than half a century and which is toxic to other Fundulus. The population also tolerates high loads of parasites (helminths and others) which are rare or absent in other populations. By studying this and control populations living in more benign environments the authors found amino acid substitutions which tend to be concentrated in different parts of the antigen-binding region of the molecule. They proved that the MHC variation is driven by selection, not drift. The first step in applying this technique in other situations, e.g. searching for selectable QTLs in an aquaculture broodstock, would seem to be finding a population that is unusually well adapted to the targeted stress.

We would like to know what the best genetic management strategy is: selection for homozygosity of particular MHC alleles, or selection for MHC diversity per se. The answer is important both to conservationists and to geneticists who hope to profit from the development of proprietary ‘super breeds’ for aquaculture. Evidence from other organisms (e.g. mice; Penn et al., 2002) suggests that it probably depends on the variety and timing of challenges anticipated from pathogens. Optimal selection strategies for populations growing in extensive and ‘biosecure’ aquaculture systems may be even more different than we thought. MHC diversity considerations may become crucial, both practically and politically, in the design of captive breeding and aquaculture genetics programs (Arkush et al., 2002).

**Diversion of resources away from growth and into reproduction**

Early-maturing male salmon and trout are a problem for aquaculture because male fish are physically unappealing and their growth slow. Age-at maturation is genetically the same trait in both sexes in salmon, judging by the strong genetic correlation between the males and females for both age of maturation and weight (Kause et al., 2003). Thus it will not be easy to develop a strain in which males mature late but maturation of females is unchanged. The heritability of both traits is sufficiently high, though, that selection for late maturation of both sexes should work if performed on either sex.

There is an analogous problem in tilapia with the difference that the female, not the male, diverts resources towards reproduction at the expense of growth. An ingenious genetic work-around for sexual dimorphism in tilapia has been found and is achieving considerable commercial success. As described by Mair et al. (1997), the procedure involves five preparatory generations of progeny testing and hormonal sex reversal, both male-to-female and the reverse. The final result is a set of YY ‘supermales’ which, when mated with normal XX females, produce offspring which are nearly 100% normal XY males. The YY supermales have a few female offspring, however, presumably because of the multifactor sex determination in this species. YY male breeders are now used commercially in many places to generate grow-out populations that consist entirely...
of genotypically normal XY males. The lack of females contributes to uniformity and more rapid growth and also stops unwanted reproduction in aquaculture ponds.

The only major problem with the above procedure is that it interferes with selection for traits such as growth rate (in the YY male donor line, at least) because of the five generations of preparation. The lag could be reduced considerably if the genotypic sexes could be identified without progeny testing.

There is reason to hope that commercially useful sex-specific markers can be found in tilapia. Harvey et al. (2002) report the development of *in situ* hybridization probes to identify sex-specific sequence differences in the long arm of chromosome 1 of the tilapia species *Oreochromis niloticus*. The binding difference between the probe sequences from X and Y chromosomes is small, which is not surprising. The authors comment that “Only limited sequence divergence between the X and Y chromosomes would be expected as YY individuals can develop into males or, if hormone treated, females that are both viable and fertile, although growth and survival rates are somewhat lower in YY than XY males.... This suggests that only a very limited loss of function can have occurred in Y-linked genes and that sequence differences between the X and Y chromosomes are largely confined to non-coding regions.”

Lee et al. (2003) used bulked segregant (BS) analysis to search for microsatellite marker genes associated with phenotypic sex in tilapia. (In BS analysis DNA from many individuals with the same phenotype is pooled and compared to a pool of DNA from a contrasting phenotype. The contrast here was male v. female phenotypes.) Ten markers were found, all on linkage group 8, which is therefore the (or a) putative Y-chromosome. The linkage of two markers with the sex-determining region was so tight that the sex of offspring of two families was correctly predicted 95% of the time. Unfortunately the markers were not linked to sex in the third family, which shows that we are still some distance away from using markers instead of progeny testing in the commercial production of tilapia YY-supermales.

The existential dilemma of aquaculture farmers

Farmers beset with problems of cost, price and risk are faced with a dilemma because ignoring genetics is likely to be just as costly as paying for it. Practical decisions about genetics must be made every time fish or shellfish are stocked. Are these breeders the best available? Are they even average? Would one of the breeds promoted on the worldwide web grow well enough to stave off bankruptcy?

The application of modern genetics is often listed as one of the highest priorities in aquaculture research and development agendas. Nevertheless, farmer associations and government agencies are slow to invest in ‘big genetics’, as has often been noted and lamented by professional geneticists. This is not because ‘big business’ is attempting to direct public-sector research towards its own interests, as it is reputed to have done during the development of scientific agriculture (Kloppenburg, 1988). Individual farmers just seem to prefer activities that they can do themselves on their own farms.

The question a farmer asks about programs that promise to provide ‘super fish’ or ‘super shrimp’ is which program will bring the largest economic benefit to his own farm. As indicated earlier, modern genetics has shown itself capable of producing genetic productivity gains measured not by increments but by multiples of the existing standard. Every time a farmer stocks or re-stocks his farm he realizes the existential horror of his predicament: the available genetic information is irrelevant and incomplete in its most crucial practical aspects; doing nothing about genetics is also taking action; the appropriate emotions for the practical person are anguish and dread.

When a new agricultural technology is introduced everyone involved can be considered either a beneficiary, a bystander or a victim. The immediate beneficiaries are easy to identify. Professional geneticists (some of them) will benefit from being the heroes of the technology and so will their research sponsors. The owners of the technology when it is successfully commercialized will also benefit. The fish-eating public will, eventually, be able to buy fish at a lower price.

Some farmers - the early adopter farmers - will also obtain a competitive commercial advantage which benefits them in the short term. The same competition, however, victimizes follow-on farmers in the short term. Simple economic theory predicts that farm-gate prices will go down, production will be up and farmers will become increasingly dependent on technology over which they have no control, just as in modern terrestrial agriculture (Allen, 1984; Kloppenburg, 1988; Weller, 1999). The owners of the technology and the fish-eating public will be sharing the benefits of the higher productivity
permitsd by the new technology and farmers will be in the same cost-price squeeze as they were before - essentially, bystanders.

If a new strain of fish or shellfish is available to all producers, market competition occurs and a new equilibrium price is reached at the new and lower production cost which, however, now includes whatever extra fees the producers have to pay for the new strain. It will of course benefit the technology developers to make their technology universally available – for a price - and not restrict it to a few producers. The benefits of the increased production efficiency accrue to the public, which may pay less for fish, and to the sellers of the technology. Competition for market share again requires producers to reduce their price and profits to the lowest tolerable level.

The above reasoning leads to the conclusion that farmers who want to benefit from purveyors of advanced technology should try to become early adopters and possibly insist on some sort of exclusive franchise arrangement to protect their competitive advantage as long as possible. The farmer should also attempt to obtain sole or part ownership of the technology in order to continue benefiting as bioeconomic equilibrium is approached. The developers of the technology will be anxious to attract early adopters for promotional reasons and the leverage this gives the farmer might be translated into franchises or ownership.

It should be emphasized that there is a divergence of interest between the developers of the technology and the farmers who use it. The former group wishes to maximise the spread of the new technology, for reasons of professional prestige (including institutional prestige in the case of universities, governments and international development agencies) as well as commercial gain.

The dilemma of aquaculture genetics companies

James (2000) recently distinguished between three types of genomics companies: product providers, information providers and technology providers. Although he wrote about companies that are working on human health issues, his analysis applies equally well to genetics in aquaculture. Companies in these three areas are now racing for primacy and moving into each other’s commercial strategy space. The question which interests Mr. James is how to invest money. The question which interests aquaculturists is how to bet the future of the fish farm, since a bet on genetics must be made.

If we apply James’s analysis to aquaculture genetics we conclude that companies which provide proprietary products like vaccines or genetically improved broodstock and fingerlings can potentially make the highest profit but also experience the highest risk, in particular the risk that someone else will develop a product which is cheaper or more effective.

Purveyors of aquaculture genetic information about genomic sequences, markers and maps are mostly but not entirely in the public sector. The opportunity to generate value from proprietary information about QTLs, pathogens and broodstock genotype-environment interaction in aquaculture is not being ignored, however. The commercial risk to information suppliers is that their proprietary information will become ‘commoditised’ and freely available. Some people are even making a moral crusade out of the public right to raw genetic data. Spider Robinson neatly summed it up in the Toronto Globe & Mail on 18 March, 2000: “It’s as though an explorer took the first photo of a zebra - then claimed ownership of zebras, the concept of stripedness, and anything else substantially zebraic in nature”.

Both in human genomics and in aquaculture genetics there are companies that develop technology for use by other companies e.g. for on-farm broodstock improvement. (My own consulting company falls into this category.) James notes that such companies “though in some ways offering the lowest risk for investors, are always in danger of becoming generic or outdated as new ways for tackling a problem are developed.”

Given the entirely rational preference that farmers have for technologies over which they have sole ownership, professional geneticists could usefully focus more effort on developing high- as well as low-technology procedures that can be applied on a small scale. Farmer-breeders would then have more scientific support and information when choosing how to act at the farm level. They might then feel more comfortable in their existential dilemma where any procedure for choosing breeders, even random choice, has genetic consequences. Such procedures would enable individuals to develop proprietary breeds on their own farms and should speed up the application of modern genetics to aquaculture.
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Feeds for the future: the importance of better broodstock and larval nutrition in successful aquaculture

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Introduction

The future of aquaculture production, as in other animal production systems, is towards greater control of the physical, chemical and biological variables surrounding the production system. The trend from collection of wild fry to the development of hatcheries for fry production, from the use of wild spawners to the use of maturation systems and ultimately closing the reproductive cycle to use domesticated and selected stocks is clear. At the same time, there is an increasing trend towards production efficiency and cost-effectiveness, not least in the hatchery stage, particularly where the supply – demand situation progresses from quantity production to quality production. These trends are becoming more evident in all forms of commercial aquaculture.

Broodstock and larval nutrition are key elements underpinning this progress towards greater control and domestication. However, there is substantial work to be done if rearing of aquaculture species is to approach the level of control and understanding which are evident in the poultry, swine and ruminant sectors. This paper gives a brief introduction to some of the key areas and issues in broodstock and larval nutrition. Constraints of time and space mean that the paper is largely confined to crustacean, and particularly, penaeid shrimp culture. However, many of the same gaps are found when looking at the broodstock and larval nutrition of most fish species.

Broodstock nutrition

Research into shrimp broodstock nutrition is gaining importance with the increasing use of domesticated and genetically selected stocks for aquaculture. Also, given the high cost of feed in a captive broodstock maturation facility, estimated at around 50% of total cost, as well as a heavy reliance on fresh feed items such as squid, mollusk meals and polychaete worms which may vary in quality and availability, there is a demand for feeds specifically formulated for use in maturation diets. It has also been pointed out (Doyle, personal communication) that the development of genetically improved lines of shrimp may benefit from a simultaneous development of feeds specifically tailored to individual strain requirements.

During penaeid shrimp maturation, nutrient reserves, mainly from the hepatopancreas, are mobilised to support ovarian and testicular maturation, gametogenesis and vitellogenesis (Harrison, 1990; 1997). Tissue reserves in the hepatopancreas can be depleted rapidly so that the diet becomes the most important contributor of nutrients to the developing egg. This is particularly true when, as is the case in most captive maturation facilities, eyestalk ablation is used to accelerate the process of gonadal maturation. The hormonal and metabolic changes that come around during such forced maturation may take place when the nutrient reserves are insufficient to support rapid ovarian development placing an even larger burden on the diet as a source of essential nutrients.

The nutrition of penaeid shrimp broodstock has recently been reviewed (Wouters et al., 2001a). The authors recognize that there is a dearth of information in the scientific literature on the subject, possibly due to the expense and complexity of running sufficiently rigorous nutrition experiments on shrimp broodstock. In addition, much of the research has been carried out with fresh feeds, either alone or in combination with formulated diets or dietary supplements.
LIPIDS

Due to the importance of lipids in crustacean maturation, much of the work carried out to date has focused on this aspect, particularly the requirement for highly unsaturated fatty acids (HUFA) and phospholipid. During maturation, lipids are mobilized from the hepatopancreas in many species and dietary lipids rapidly processed for transport to the developing ovaries.

Total lipid does not appear to be important although Wouters et al. (2001b) reported that excessively high total lipid in the diet had an adverse effect on ovarian maturation and feed consumption, possibly due to satiation. However, the average lipid level in commercial broodstock diets (10%) appears to be around 3% higher than in grower feeds used in commercial culture ponds.

Highly unsaturated fatty acids (HUFA), especially 20:5n-3 and 22:6n-3, are abundant in ovarian tissues and are believed to be an important component of live and formulated maturation diets. Diets deficient in n-3 HUFA have been found to have a negative effect on ovarian development, fecundity and egg quality (Teshima et al., 1988; Alava et al., 1993; Xu et al., 1992; 1994; Cahu et al., 1994; Wouters et al., 1999a).

Arachidonic acid (20:4n-6; AA) has been detected at high levels in the ovaries of wild shrimp and is also abundant in some of the best fresh feed items such as polychaetes (bloodworms), clams and mussels (Harrison, 1997; Wouters et al., 2001a). The n-6 HUFA are known to be precursors of the prostaglandin hormones, which act in reproduction and vitellogenesis. According to Wouters et al. (2001a), formulated maturation diets appear to be deficient in AA as well as relatively low eicosapentanoic acid (EPA) levels. It has been proposed that the ratio of n-3 to n-6 levels in the diet is important (Lytle et al., 1990) and that it should be around 2–3 to 1 (Ravid et al., 1999; Wouters et al., 1999b).

Phospholipids, mainly phosphatidylcholine and phosphatidylethanolamine appear to be predominant in the shrimp ovary and there seems to be a requirement for phospholipids in the diet. Several studies (Alava et al., 1993; Cahu et al., 1994; Ravid et al., 1999; Wouters et al., 1999b) have demonstrated the effects of phospholipid levels in the diet and it has been suggested that broodstock diets should contain more than 2% phospholipid to ensure that 50% of total egg lipid is in this form (Cahu et al., 1994).

Cholesterol is the precursor of steroid hormones and it is known that shrimp have a requirement for cholesterol in the diet. Cholesterol is stored in the hepatopancreas and is mobilized during maturation. The role and mobilization of cholesterol during shrimp maturation has been reviewed by Harrison (1990). Some of the live feed organisms used in maturation diets have relatively high cholesterol levels (e.g. squid, clams) although to date there has been limited research into the effects of dietary cholesterol on maturation and reproduction (Harrison, 1997; Wouters, 2001).

During maturation, the level of triacylglycerides (TAG) in the ovaries increases as they are incorporated into the egg and decrease after spawning (Ravid et al., 1999; Wouters et al., 1999b). Triacylglycerides appear to be the principal energy source in eggs and nauplii and their importance in reproduction, and egg and postlarval quality has been shown (Palacios et al., 1998; 1999).

PROTEIN AND AMINO ACIDS

Maturation is a time of intense protein synthesis and it is likely that the requirement for protein is higher at this time (Harrison, 1990; 1997). Wouters et al. (2001a) report that the protein content of formulated diets in their studies was around 50% but that this was still low compared to the level in fresh feeds. Detailed studies into protein requirements for shrimp broodstock are still lacking and it has been proposed that the amino acid profiles should mimic those found in fresh feeds (Deshimaru, 1982).

Some studies have shown changes in protein content of the ovaries associated with egg development and spawning, and with spawning success. Animulkar (1980), reported in Harrison (1997), found an increase in ovarian protein levels associated with ovarian development followed by a sharp decrease after spawning in the shrimp Paratelphysa hydrodromaus and several authors have noted a similar increase in farmed penaeid shrimp (Read and Caulton, 1980; Castille and Lawrence, 1989). A marked difference has also been noted in the protein content of the hepatopancreas and ovaries of wild and domesticated females of Litopenaeus vannamei with good repeat spawning performance which have been found to have significantly higher protein content than females with poorer spawning performance (Palacios et al., 2000).
CARBOHYDRATES

Carbohydrates do not appear to be essential for shrimp broodstock diets although Palacios et al. (1998; 1999) related egg glucose levels with larval quality and broodstock condition. Carbohydrates can be used as cost-effective ingredients to contribute to glycogen accumulation in the hepatopancreas (Harrison, 1997) as well as providing other benefits in the broodstock diet, acting as binders and possibly playing a role in transport of nutrients in the hemolymph (Harrison, 1997).

VITAMINS AND MINERALS

Detailed vitamin and mineral requirements for shrimp broodstock diets are relatively unknown with only a few studies on vitamins A, C and E. Alava et al. (1993) found that ovarian maturation was slower when fed a diet deficient in either vitamins E, A and C. Vitamin E appears to be important in crustacean broodstock nutrition. Chamberlain (1988), reported in Harrison (1997), found a correlation between vitamin E deficiency and the percentage of abnormal sperm in Litopenaeus setiferus and Cahu et al. (1991) found an improvement in hatching rate with increasing dietary vitamin E correlated to increasing α−tocopherol levels in the egg. Wouters et al. (1999b) found a similar correlation to that observed by Cahu et al. (1991) between spawn and hatch quality with α-tocopherol levels in wild spawners and nauplii of L. vannamei. They found that mature ovaries and nauplii contained higher levels of α−tocopherol than immature and spent ovaries. Harrison (1997) also speculated that vitamin E in the egg yolk may also act as a natural antioxidant.

Work conducted by Fisher and Kon (1958), reported in Harrison (1997), suggested the importance of dietary vitamin A due to its accumulation in the ovaries of crustaceans during maturation. Vitamin C content of eggs of Fenneropenaeus indicus are also influenced by the levels in the diet, and high hatching rate has been related to high ascorbic acid levels in the eggs (Cahu et al., 1995). Harrison (1997) assumes that vitamin D is important in broodstock diets due to its probable role in calcium and phosphorus metabolism in crustaceans.

Harrison (1990) discussed the possibility that mineral deficiencies or imbalances could have a negative impact on crustacean reproduction and may play a role in oocyte resorption, reduction in reproductive performance and egg quality. Studies into mineral requirements are rare due to several complications (Wouters et al., 2001a). Where studies have been conducted, diets were formulated with mineral mixes with added calcium, phosphorus, magnesium, sodium, iron, manganese and selenium (Chamberlain, 1988; Marsden et al., 1997; Mendoza et al., 1997; Xu et al., 1994).

Spent broodstock of L. vannamei had lower levels of calcium and magnesium in the muscle and lower magnesium levels in the hepatopancreas (Mendez et al., 1997), possibly due to a combination of dietary deficiencies and losses through moulting and transfer to the eggs. Copper also decreased in the hepatopancreas, possibly through transfer to the ovaries, although it increased in the muscle tissue. It is clear that more studies need to be undertaken into mineral nutrition in broodstock diets.

CAROTENOIDs

Crustaceans cannot synthesise carotenoids de novo, and a dietary source of these pigments is required. During sexual maturation, most crustaceans accumulate carotenoids in the hepatopancreas and during vitellogenesis, these are transported in the hemolymph as carotenoglycolipoproteins to accumulate in the eggs as part of the lipovitellin protein. Dall (1995) found that free astaxanthin levels in the developing ovaries of Penaeus esculentus increased from 2 to 34 ppm and in the digestive gland, from 20 to 120 ppm.

Carotenoids, especially astaxanthin, are strong antioxidants and probably play a role in protecting the broodstock nutrient reserves and developing embryos from oxidation (Dall et al., 1995; Merchie et al., 1998). It has also been suggested (Harrison, 1997) that they act as pigment reserves in the embryos and larvae for the development of chromatophores and eyespots, and as a vitamin A precursor (Dall, 1995).

Wyban et al. (1997) noted a decrease in nauplius quality with successive spawns associated with a loss of pigmentation in the ovary of L. vannamei. Addition of paprika, an inexpensive source of carotenoids, to the fresh diet at a rate of 2% (2 g paprika per 100 g squid meat), resulted in a significant improvement in nauplius quality (measured as survival to zoea 2 stage).

Pangantihon-Kühlmann and Hunter (1999) found that astaxanthin supplementation (50 mg/kg) of the diet resulted in increased egg production in Penaeus
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monodon but could not demonstrate any benefit of astaxanthin supplementation on either hatching rate or metamorphosis to zoea 1 stage.

MISCELLANEOUS FACTORS

**Hormones**

It has been suggested that some of the more successful live feed organisms may provide benefits through the provision of hormones or their precursors. Naessens et al. (1997) speculated that part of the reason for the success of reproductive adult *Artemia* biomass supplementation of the diet of *L. vannamei* broodstock could be due to the presence of specific hormones or analogous peptides in the *Artemia* that provoked a response in the shrimp. Bloodworms used in maturation have also been found to contain methyl farnesoate, an ecdysone hormone that has been shown to increase reproductive performance in the spider crab *Libinia emarginata* (Laufer et al., 1987), *L. vannamei* (Laufer et al., 1997), *P. monodon* (Hall et al., 1999) and the crayfish *Procambarus clarkii* (Laufer et al., 1998). In *P. clarkii*, the hemolymph titer increased from basal levels during early vitellogenesis, peaked during mid-cycle and then returned to basal levels when the ovaries were in late vitellogenesis.

**Nucleotides**

Nucleotides, the basic building blocks of nucleic acids, are recognised as important elements in mammalian nutrition especially during periods of rapid growth or physiological stress (Uauy, 1989; 1994; Barness 1994; Van Buren, 1994) and also appear to play a key role in the immune system. Traditionally, nucleotides have not been considered essential nutrients although de novo synthesis and salvage pathways are thought to be costly processes in metabolic terms. Several studies have demonstrated that dietary sources of nucleotides can have beneficial effects and the term ‘conditionally essential’ has been used to describe their role in nutrition (Carver and Walker, 1995). Exogenous sources of nucleotides are thought to optimise the functions of rapidly dividing tissues, such as those of the developing embryo and young, and the reproductive and immune systems.

Most aquaculture diet ingredients of animal and plant origin contain nucleotides although there are differences in the concentration and availability. Nucleotide content is particularly high in ingredients such as fish solubles, animal protein solubles, fish meal, legumes (adenine is particularly high in black-eyed peas), yeast extracts and unicellular organisms such as yeasts and bacteria that are rich in RNA or DNA (Carver and Walker, 1995; Devresse, 2000). Devresse (2000) noted that the low digestibility of whole yeast compared to yeast extract may be related to the protein (nitrogen) solubility as yeast extract has much higher protein solubility than whole yeast. He also noted that, although fish solubles are highly digestible, they leach easily in water affecting availability.

Reproduction and egg development have a high requirement for RNA and DNA and it may be expected that increasing the availability of nucleotides in broodstock diets may have a beneficial effect on egg development. Recently, research has demonstrated the effect of a nucleotide-enriched diet for broodstock nutrition in aquaculture (Gonzalez-Vecino, 2002; Gonzalez-Vecino et al., 2003). Nucleotide enrichment of broodstock diets for Atlantic halibut (*Hippoglossus hippoglossus*) and haddock (*Melanogrammus aeglefinus*) resulted in a general trend towards better spawning performance and egg quality with the nucleotide diet. Total egg yield was 30% higher in the halibut fed with the nucleotide diet and the relative fecundity, mean egg density, hatching rate and survival of yolk-sac larvae were also significantly improved. Haddock fed on the nucleotide-enriched diet also had significantly higher fertilization and hatching rates. To date, no work has been published on nucleotide supplementation of broodstock diets for shrimp but it would be interesting to conduct some trials to determine if broodstock diets enriched with nucleotides might offer similar benefits in shrimp maturation and breeding. Similarly, the potential for nucleotide supplementation of diets for shrimp larvae should also be investigated.

**Larval feeds**

Typically, marine larvae are fed with live feeds such as algae, zooplankton, rotifers and *Artemia*. In some cases, especially with rotifers and *Artemia*, the fatty acid profile is inadequate (Léger et al., 1986), especially with regard to the HUFA profile. The practice of enrichment has been developed as a means of overcoming this nutritional deficiency.

Enrichment usually involves enhancing the docosahexaenoic acid (DHA) and EPA content of the natural feed through ‘bioencapsulation’. This
involves feeding the live organism with a DHA/EPA enriched formulation to boost the levels in the tissues and then feeding the enriched organism to the larvae. In the case of shrimp larvae, the use of enriched Artemia is restricted to the post-larval stages due to the size of the first feeding instar stages of the Artemia (Sorgeloos et al. 1998).

FORMULATED FEEDS

Although live feeds provide an excellent source of nutrition, there are several drawbacks associated with their use. Algal cultures require considerable expertise to maintain them in peak nutritional condition and facilities for their mass production can be expensive to operate. Rotifers also require considerable expenditure in time and effort to maintain, especially if they, in turn, need to be provided with live feed. Live Artemia nauplii suffer from inconsistent supply and quality as they are obtained from cysts collected in the wild environment. The bulk of cysts come from the Great Salt Lake in Utah in the US where annual fluctuations have been shown to cause wide fluctuations in yield. As a result, price and quality can vary unpredictably (D’Abramo, 2002).

Such problems with live feeds have led to the development of diets specifically formulated for their replacement. However, the development of formulated larval diets to completely replace live feeds has been an elusive goal, despite considerable effort (Langdon, 2003). The difficulties inherent in providing a complete nutritional package in a sufficiently small particle to be ingested and digested by the small larvae of many marine species are clear. Loss of nutrients from such diets can be rapid and result in loss of nutritional value and fouling of the culture medium and should be used to determine the effectiveness of microparticulates as nutrient delivery systems. On the other hand, provision of a sufficiently impermeable coat to prevent leaching may result in poor digestibility and availability of the nutrients to the developing larvae. Microbound and cross-linked protein-walled capsules may be used to deliver lipids and high-molecular weight, water-soluble nutrients such as proteins and carbohydrates whereas lipid-based particulates, including liposomes, could be useful in delivering low-molecular weight, water-soluble nutrients, such as amino acids and water-soluble vitamins.

Although the use of formulated larval feeds as partial replacements for microalgae is common in commercial hatcheries, total replacement of algae has proved to be more difficult. Complete replacement has only been achieved using ocean quality seawater that is partly filtered to retain the natural bacterial community (Ottogali, 1991; 1992) and reports of complete replacement in commercial hatcheries appears restricted to those located in the oceanic waters of the Pacific islands (Chim, 2003). Alabi et al. (1997) also showed that total replacement requires the establishment of a balanced bacterial community from either the filtered seawater or following conditioning by microalgae. As a result Jones et al. (1997; 1998) suggested the inoculation of a single dose of live algae (SDLA) before use of artificial feeds to condition hatchery water when it is taken from coastal water of variable bacterial quality.

Wouters and Van Horenbeek (in press) summarize the various types of commercial larval feeds available in the market. These include microbound diets, flakes, granulated feeds, microencapsulated feeds, liquid feeds (lipid-walled capsules).

Microbound feeds are bound using a variety of different binders and produced as a small particle, or as a pellet, cake or flake, which is then crumbled to the appropriate size. They are inexpensive to produce but leach rapidly. Crumbles are usually used during postlarval stages.

Flakes are commonly used in Asia and the Americas. Dietary ingredients are added to water to obtain a dense soup. An appropriate binder is added and the resulting suspension is sprayed onto a steam-drum dryer. Temperatures can exceed 100°C. and significant nutrient loss can occur unless passage times are kept short. Large flakes can be crushed and passed through an appropriate mesh screen immediately prior to use. They are generally used for the postlarval stages of the shrimp.

Granulated feeds are produced using liquid binder and water sprayed onto the feed mix, resulting in granules with a raspberry-like structure.

Microencapsulated feeds have an outer coat (capsule) that retains the ingredients inside the particle. They can be designed to have a slow release of the material or to totally prevent leaching of water-soluble nutrients. Some techniques encapsulate using a cross-linked protein-wall that can be digestible yet capable of withstanding drying.

Liquid feeds are essentially a slurry of particles in a suspension medium. Although expensive, they are claimed to cause less fouling and can be continuously dosed into larviculture tanks using peristaltic pumps.
CONSIDERATIONS IN DEVELOPMENT OF FORMULATED LARVAL FEEDS

The nutrition of marine larvae involves an understanding of the behavioural, mechanical and physiological processes of feeding in the target animal. These are likely to be very different in the larval stages compared to the adult form. Feeding habits in many species show a distinct change as the larvae develop. In penaeid shrimp, many species change from a primarily herbivorous diet in the zoea stages to a more omnivorous diet in the postlarval stages (Lemos and Phan, 2001). During the postlarval and early juvenile stages, further changes may involve a switch to a more carnivorous or detritivorous diet depending on the species. There are also changes from a planktonic existence to a benthic one and from a filter feeder to an active predator to be considered, all within a few days of hatching.

One of the key considerations is the development of the gut structure and function. Larval crustaceans have a simple gut structure, which gradually becomes more complex. The physiology of the gut and gut enzymes also changes and, since transit times may be quite short (Jones et al., 1997; Jones and Kurmaly, 1987), designing a nutritious, easily digestible diet is a challenge.

Digestive physiology of marine larvae

The development of the digestive system of marine larvae plays a fundamental role in larval nutrition. In particular the development of gut function and ontogenetic changes in enzyme function are of critical importance.

The structure of the larval gut in crustaceans has been studied in only a few species (Factor, 1981; Lovett and Felder, 1989; 1990a; 1990b; Abubakr and Jones, 1992). The development of gut structure appears to follow similar patterns in most of the species studied. The gut of early larval stages of penaeids is relatively simple and the gastric mill and hepatopancreas are either not present or not yet functional. Feed is mixed with enzymes through expansion and contraction of the midgut gland and there is free fluxing of food between the midgut gland and the midgut, especially in time of food scarcity (Lovett and Felder, 1990b). As the larva grows, the hepatopancreas proper develops, the lobes increasing in number and elongating during the mysis stages. At this point, the rudimentary gastric filter begins to develop. The postlarval stages are marked by the rapid development of the hepatopancreas, which increases in size and complexity, and the development of the gastric mill and gastric filter to the adult form.

Until the gastric mill becomes functional, the shrimp larvae are restricted to feed items that are relatively easily digestible. In the case of the herbivorous stages, they generally filter the feed from the water and digestion takes place in the gut. As the appendages and mouthparts become stronger and more complex, larger feed particles and live prey are held and torn apart before ingestion, rendering them more easily digestible.

There has been relatively little research into the digestive enzymes of crustacean larvae. Jones et al. (1997) noted that larval enzymes differ in range and level of activity from those that are present in adults and that there exist species differences which may relate to the feeding strategy of the larvae. There are also changes in enzyme activity patterns between different larval stages and, overlaid on these, the possibility of diet-induced changes in enzyme levels.

In a brief review of research into enzyme activity of decapod larvae, Jones et al. (1997) reported that all appeared to have strong protease activity, predominantly trypsin, non-specific esterase activity and amylase. In the few reports available, pepsin appears to be absent in larval and most adult decapods (Glass et al., 1989) although it has been reported in the freshwater prawn, Macrobrachium rosenbergii (Lee et al., 1980). Similarly, lipase activity was only reported in larval stages of the black tiger prawn, Penaeus monodon and the lobster, Homarus americanus although this may be open to debate. Collagenase, elastase and chymotrypsin all seem to be rare or absent in larval stages.

Changes in enzyme activity patterns with larval stage have been clearly demonstrated in penaeids (Lovett and Felder, 1990a, b; Glass et al., 1989; MacDonald et al., 1989; Ribeiro and Jones, 2000; Ngamphongsai, 2000; Puello-Cruz et al., 2002). MacDonald et al. (1989) noted that the general pattern of amylase, protease and lipase in P. monodon larvae was similar with a maximum of enzymatic activity at zoea 3, possibly coinciding with the change from predominantly filter feeding to mechanical digestion. They also noted a minimum level of all three enzymes in the early postlarval stage, similar to that noted by Lovett and Felder (1990a) (the so-called ‘enzyme crisis’), which was attributed to a change in gut development associated with changing feeding habits as the postlarvae become more benthic. This coincides with a critical period in postlarval development when
poor postlarval condition and high mortality may be observed in commercial hatcheries. Puello-Cruz et al. (2002), on the other hand, found that trypsin levels in *Litopenaeus vannamei* larvae were significantly highest at the Zoea 1 stage, and declined thereafter to the PL1 stage. They also noted that trypsin content in Zoea 2 and Zoea 3 stage larvae feeding on *Artemia* was significantly lower than in those feeding on algae and suggested that *L. vannamei* may be physiologically adapted to transfer to a more carnivorous diet during the Zoeal stages than more herbivorous species such as *P. monodon* or *P. indicus* (Ngamphongsai, 2000). This accords well with commercial hatchery observations that *L. vannamei* is capable of feeding on *Artemia* from the Zoea 3 stage whereas *P. monodon* larvae do not become fully capable of feeding on live *Artemia* until at least mysis 2 or 3. Changes in the sequence of enzyme activity consistent with a carnivorous diet were also noted in early larval stages of *Macrobrachium rosenbergii* (Kamarudin et al., 1994).

Several authors have suggested that fish larvae may benefit from the assistance of exogenous digestive enzymes of their live food organisms, either through autolysis or by the action ofzymogens that activate larval endogenous digestive enzymes (Kolkovski et al., 1993; 1997; Munilla-Moran et al., 1990) and the same may hold for crustacean larvae.

**LIPIDS**

Much work on larval feeds has centred on the lipid requirement, in particular the requirement for highly unsaturated fatty acids (HUFA) and phospholipids. The n-3 highly unsaturated fatty acids (HUFA) docosahexaenoic acid (22:6n-3, DHA) and eicosapentaenoic acid (20:5n-3, EPA) are essential for normal growth and development of many species of marine fish and crustaceans (Sargent et al., 1997; Jones et al., 1997; Suprayudia et al., 2004). Marine fish are also unable to synthesize arachidonic acid (20:4n-6; AA).

Quantitative requirements for essential fatty acids have been rarely reported for larvae of penaeid shrimp. It has been suggested (Kanazawa et al., 1979, in González-Félix and Pérez-Velazquez, 2002) that 1% n-3 HUFA in the diet could be considered as a minimal value for postlarvae although Chen and Tsai (1986) indicated a requirement for HUFA at 0.5-1% of the diet for *P. monodon* and Xu et al. (1994) suggested a requirement of between 0.7% and 1% of the diet in *Fenneropenaeus chinensis*.

The dietary effects of phospholipids on fish and crustacean larvae have been reviewed by Coutteau et al. (1997). Although there appears to be little doubt that crustacean larvae can synthesise phospholipid from HUFA, the addition of dietary sources of phospholipid has been shown to be beneficial, possibly through enhancing the absorption of dietary cholesterol and triacylglycerols (Jones et al., 1997). Gonzalez-Félix et al. (2000), for example, demonstrated that *L. vannamei* postlarvae fed diets containing phospholipid demonstrated improved growth and enhanced muscle phosphatidylcholine and phosphatidylethanolamine concentration. Coutteau et al. (1996) however, showed that the other phospholipids in lecithin could not compensate for phosphatidylcholine deficiency in the diet of postlarval *L. vannamei* and that, as dietary phosphatidylcholine increased, significantly higher levels of total HUFA, 20:1n-9 and 20:5n-3 were present in the shrimp.

In experiments carried out with larval *P. japonicus*, optimal metamorphosis was obtained with diets containing 15-30 g/kg soybean phosphatidylcholine and the lower level (15 g/kg) was more beneficial to postlarvae (Camara et al., 1997). Results also suggested that there was no requirement for phosphatidylethanolamine or phosphatidylinositol in the presence of adequate dietary phosphatidylcholine. The phospholipid requirement of crustacean larvae from this study appears to be within the range of 1-3% given by Coutteau et al. (1997) as the range for most marine larval species and agrees with the earlier figure of 3% given by Kanazawa (1990).

As with adult crustaceans, larvae are unable to synthesise cholesterol and have an absolute requirement for cholesterol in the diet (Teshima et al., 1983).

**PROTEIN AND AMINO ACIDS**

The optimal dietary protein level in a larval diet can be expected to vary with species, larval stage (Durruty et al., 2002), protein source, digestibility (Le Vay et al., 1993) and amino acid composition. Kanazawa (1990) recommended protein levels in larval feeds between 23% and 57%. Durruty et al. (2002) reported differences in protein requirement according to the larval stage of *L. vannamei* and *L. setiferus*. They estimated that protein requirement increased from 30% in the Zoea stages up to 50% or 60% for mysis stages. Data on optimal protein:energy ratios
or amino acid profiles for larval shrimp feeds have not been reported (Wouters and Van Horenbeek, in press).

The essential amino acids for shrimp are thought to be methionine, arginine, threonine, tryptophan, histidine, isoleucine, leucine, lysine, valine and phenylalanine (Akiyama, 1992). There is no reason to believe that shrimp larvae have any specific amino acid requirement that differs from the adult shrimp (Jones et al., 1997). Although no single protein source appears to satisfy the complete requirements for amino acids (Wouters and Van Horenbeek, in press), Cahu (1999) stated that lower nutritional value protein sources can be fortified by the addition of essential amino acids (Cahu, 1999).

CARBOHYDRATES

As with adult crustaceans, there appears to be no specific requirement for carbohydrates in the diet. Carbohydrates can be used to reduce feed costs through protein or lipid sparing and are frequently used as binders in larval feeds.

VITAMINS AND MINERALS

Fat-soluble and water-soluble vitamins as well as carotenoids are essential for shrimp larvae (Wouters and Van Horenbeek, in press). Kanazawa (1986; 1990) determined the vitamin requirements for *P. japonicus* (Table 1). These levels provide a practical baseline, but Kanazawa (1990) also admits that they may be affected by leaching of the vitamins from the test diet. In practice, formulated feeds for shrimp larvae will contain a complete vitamin and mineral premix as used in feeds for older shrimp.

Table 1. Vitamin requirements of *P. japonicus*.

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>(mg/kg of dry diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamin HCl</td>
<td>4</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>8</td>
</tr>
<tr>
<td>Pyridoxine HCl</td>
<td>12</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>40</td>
</tr>
<tr>
<td>Biotin</td>
<td>0.2</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>600</td>
</tr>
<tr>
<td>Inositol</td>
<td>200</td>
</tr>
<tr>
<td>Na-ascorbate</td>
<td>1,000</td>
</tr>
<tr>
<td>Tocopherol</td>
<td>20</td>
</tr>
</tbody>
</table>

1Adapted from Kanazawa (1986; 1990)

Kanazawa’s work on vitamin C requirements was conducted using sodium ascorbate. More recently, vitamin C has been available in the form of L-ascorbyl-2-polyphosphate. Although there is little work on larval nutrition using this form of the vitamin, work on the early postlarval stages of *L. vannamei* has shown that high dietary levels (40 mg/kg) can increase resistance to salinity stress. The benefit of elevated dietary vitamin C levels in increasing stress and disease resistance has also been noted by other authors (Kontara et al., 1997; Merchie et al., 1997; 1998).

OTHERS

Nucleotides have demonstrated much potential when fed to the young or juvenile stages of vertebrates (Carver and Walker, 1995). The benefits of adding dietary nucleotides to feeds for the rapidly developing larval stages of crustaceans would appear to be clear. However, there are no research data available on the application of exogenous nucleotides for crustacean larval culture.

Hatchery operators often use a mixture of additives in a typical feeding regime including additives such as immune stimulants or probiotic bacteria. There is still little or no scientific data to support the use of such additives in shrimp larval culture.

Conclusion

The current state of knowledge of broodstock and larval nutrition of crustaceans contains many gaps. This is partly the result of the complexity and expense of conducting research into these two areas. However, given the increasing importance of domestication in shrimp aquaculture particularly, there is a need for an increased focus on these areas. The role of nutrition in broodstock and maturation performance and in increasing larval survival and quality will be fundamental to obtaining optimal performance from domesticated stocks. Even in species where domestication is not an issue, the improvement of maturation performance and larval production remains a key goal in improving the efficiency of production systems.

It is not clear how far the goal of complete replacement of live feeds may be. It is likely that this will be attained in broodstock diets long before larval feeds. Indeed, given the complexities inherent in supplying a complete nutritional package in a small particle, it may be that this goal is never reached.
However, it may be possible to supply a range of diet particles (e.g. high lipid particles, high protein particles, carbohydrate particles etc.) that are aimed specifically at providing the right mix of nutritional elements in the culture tank that will expose the larvae to the appropriate nutritional mix. Alternatively, it may be that, as expressed by D’Abramo (2002), the complexity of the ontogeny of larval nutritional physiology may mean that technical success will be based on a compromise between the desire to provide a complete diet package and the need to strive for simplicity in formulation and manufacture.

Acknowledgements

I would like to acknowledge the debt owed to Roeland Wouters of INVE Technologies, Belgium and Prof. Patrick Sorgeloos of the University of Ghent for their kind help and provision of access to their publications for use in writing the paper.

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Feeds for the future: the importance of better broodstock and larval nutrition in successful aquaculture


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Feeds for the future: the importance of better broodstock and larval nutrition in successful aquaculture


(Boone) fed increasing levels of total dietary lipids and HUFA. Aquac. Res. 32:573-582.
European finfish culture: current status, recent advances and future perspectives

JOHN W. SWEETMAN

Ecomarine Ltd, Cephalonia, Greece

Introduction

The latest review of the state of world aquaculture from the Fisheries and Agriculture Organization (FAO) 2003 and the latest FAO Fishstat Plus statistics from 1950-2001 highlight the continuing growth of aquaculture in contributing to the total fisheries catch (Figure 1).

Aquaculture represented 5.3% of the total fisheries landings in 1970 and this had increased to 34% in 2001, i.e. 48.4 million metric tonnes (mmt) of the total fisheries landings of 142.1 mmt. The value of world aquaculture production is now estimated at 61.5 billion US$. Globally the sector has shown an average annual compounded growth rate (APR) of 8.9% since 1970 compared to 1.4% for capture fisheries and 2.8% for terrestrial farmed meat production.

Finfish production at 24.4 mmt represents 50% of the aquaculture production and over 130 major finfish species are cultured world-wide. These statistics alone emphasize the scale, complexity, rate of development and diversification of the global finfish aquaculture sector.

European aquaculture

Europe has a total aquaculture production of over 2 mmt, and of the 210 aquaculture species cultured worldwide 60 are cultured in Europe with a value of 4.6 billion US$. This means that while Europe contributes 4.4% to global production, it represents 8.2% of its total value. Recently growth from

![Figure 1. World fisheries and aquaculture production (FAO, 2003).](image-url)
aquaculture has slowed from 7.8% p.a. in the period 1980 to 1990 to 2.3% p.a. in the period 1990 to 2000.

In 2001, 1.34 mmt of Europe’s 2 mmt was attributed to finfish production and the breakdown of production among fresh, brackish and marine environments is shown in Figure 2. Salmon, trout, sea bass and sea bream account for over 1 mmt or 3 billion US$ in value. Salmon is by far the largest activity accounting for 647 thousand metric tonnes (tmt) and 1.86 billion US$. The marine and diadromous finfish species are valued at nearly 3 times the price of the fresh water species according to global FAO statistics and it is primarily in this area that European aquaculture has focused and developed (Tacon, 2003).

Table 1 shows the breakdown of the finfish species cultured in Europe and it can be seen that the largest growth is in mariculture with salmon, sea bream, sea bass and turbot. Currently the 15 countries of the European Union produce approximately 50% of the finfish total but that proportion will change with the accession of a further 10 countries later this year.

![Figure 2. European finfish production by environment (1996-2001).](image-url)

<table>
<thead>
<tr>
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<td>Tilapias</td>
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<td>200</td>
<td>200</td>
<td>246</td>
<td>180</td>
<td>200</td>
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<td>Sturgeon</td>
<td>1,285</td>
<td>1,471</td>
<td>2,022</td>
<td>2,441</td>
<td>3,083</td>
<td>3,087</td>
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<td>Eels</td>
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<td>8,696</td>
<td>9,792</td>
<td>10,536</td>
<td>10,713</td>
<td>10,187</td>
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<td>Catfish/perch/pike</td>
<td>19,321</td>
<td>16,389</td>
<td>15,437</td>
<td>19,903</td>
<td>16,583</td>
<td>14,868</td>
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<tr>
<td>Carps and cyprinids</td>
<td>175,910</td>
<td>172,620</td>
<td>180,015</td>
<td>191,236</td>
<td>197,405</td>
<td>210,667</td>
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<td>Trout</td>
<td>279,060</td>
<td>293,142</td>
<td>304,485</td>
<td>301,753</td>
<td>301,371</td>
<td>331,805</td>
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<tr>
<td><strong>TOTAL</strong></td>
<td>484,510</td>
<td>492,518</td>
<td>511,951</td>
<td>526,115</td>
<td>529,335</td>
<td>570,814</td>
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<td>Sole</td>
<td>31</td>
<td>25</td>
<td>22</td>
<td>19</td>
<td>23</td>
<td>37</td>
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<tr>
<td>Halibut</td>
<td>2</td>
<td>8</td>
<td>13</td>
<td>34</td>
<td>37</td>
<td>93</td>
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<tr>
<td>Cod</td>
<td>191</td>
<td>304</td>
<td>199</td>
<td>157</td>
<td>169</td>
<td>763</td>
</tr>
<tr>
<td>Turbot</td>
<td>2,663</td>
<td>3,041</td>
<td>3,107</td>
<td>4,113</td>
<td>4,789</td>
<td>4,959</td>
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<td>Amberjack and tunas</td>
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<td>1</td>
<td>1,959</td>
<td>3,246</td>
<td>3,686</td>
<td>4,453</td>
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<td>Misc. marine</td>
<td>740</td>
<td>1,205</td>
<td>2,153</td>
<td>2,733</td>
<td>3,031</td>
<td>2,275</td>
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<tr>
<td>Other breams and mullets</td>
<td>4,086</td>
<td>3,946</td>
<td>4,051</td>
<td>4,016</td>
<td>4,190</td>
<td>4,137</td>
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<tr>
<td>Sea bass</td>
<td>19,325</td>
<td>24,079</td>
<td>30,168</td>
<td>38,215</td>
<td>41,870</td>
<td>42,600</td>
</tr>
<tr>
<td>Sea bream</td>
<td>23,304</td>
<td>29,139</td>
<td>37,626</td>
<td>49,601</td>
<td>58,163</td>
<td>63,370</td>
</tr>
<tr>
<td>Salmonids</td>
<td>416,358</td>
<td>473,159</td>
<td>510,059</td>
<td>611,671</td>
<td>617,898</td>
<td>647,043</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>466,766</td>
<td>534,901</td>
<td>589,352</td>
<td>713,784</td>
<td>733,853</td>
<td>769,730</td>
</tr>
</tbody>
</table>

| Total European production | 951,286| 1,027,419| 1,101,303| 1,239,899| 1,263,188| 1,340,544 |

SALMON PRICE: A CONTROLLING FACTOR

Salmon prices have decreased since 2000; and in 2003 reached their lowest point ever at under 2 €/kg, with a recent average price of approximately 2.5 €/kg (Figure 3). This for many farms is at or below their production costs. Due to the importance of this species in the overall framework of the European industry, the repercussions for industry as a whole have been significant. During the same period price decreases were also being seen in the sea bass and sea bream industry with similar effects. Overall in the EU there has been a 6.5% APR in production growth but the overall price trend has been negative (-0.5% APR) versus a positive global development (FEAP).

Share prices have fallen sharply, particularly in the salmon industry; and investment confidence in aquaculture has been shaken. Pressure has been placed on the industry to further improve efficiency and productivity. Acquisitions and mergers have been the order of the day and groups are consolidating in order to benefit financially.

Significantly, with salmon being the power house of the European industry, diversification into other marine finfish species has been slowed due to the reluctance of financial institutions to invest in a variant of this troubled sector. Further, this financial pressure comes at a time when consumer awareness is focusing upon product quality, ease of product use, food safety, and traceability. This is also occurring during a period when the image of aquaculture has suffered by what the industry feels is often unfair treatment in the press.

The industry, while weakened, has responded with a positive approach. At every level the aquaculture sector is promoting transparency, cooperation and dialogue. The Federation of European Aquaculture Producers (FEAP) initiated the ‘Aquamedia’ project and this is now providing factual, truthful and interesting information about European aquaculture to the public.

In order to focus the European effort and to promote a coherent development strategy, FEAP reported to a hearing on European Aquaculture held by the EU committee of the European Parliament in October 2002. The following key issues were presented:

- Improved economic viability, long term market stability and improved profit margins within the industry are essential in order to stimulate investment and reinvestment.
- Improved access to consolidated marketing and promotional efforts would benefit the medium and smaller producers.
- The industry should within the EU operate on an even playing field with regard to imported products.
- Food safety should be a guarantee for the consumer.

![Figure 3](image-url). Cross section price for fresh whole gutted superior Atlantic Salmon, iced in poly boxes (Intrafish).
• Ingredient input quality to be provided by the feed manufacturers.
• The practical application of traceability and labeling to be implemented.
• The industry should be sustainable with regard to environmental impact and resource issues e.g. water/feed/biodiversity.
• Ensure human resources continuation through training, technology and professional entry.
• Better governance and responsibility within the industry.
• Better acceptance of aquaculture by the public.

It concluded that the long-term viability of the sector would depend upon the satisfaction of these multiple criteria and that the development of sustainable and responsible aquaculture will require coherent and viable European actions including simplified legislation and simplified licensing procedures.

Evolution of European finfish culture

The marine and diadromus finfish sector is of vital importance to the European industry and consists primarily of high valued species requiring considerable technological and managerial sophistication to culture in a sustainable manner. The critical developments that were responsible for the commercialization of these species and their subsequent diversification are outlined below. Further areas of future development and improvement required to ensure the continued development of the industry are considered in light of the rapidly evolving requirements of modern day Europe and the consumer.

While the latest FAO/FEAP statistics described above provide a fascinating insight to the development of the aquaculture industry, they obviously lack the benefit of real time information and the regional focus of development promoted by the European Union, national governments and the industrial sector. This has been achieved through supporting research and development, financial support for the establishment of the industry and by addressing marketing and consumer needs. These driving forces have provided a basis for development and diversification.

CRITERIA FOR SPECIES SELECTION

The selection of a species suitable for European aquaculture depends primarily on its market value, an understanding of its biology and the ability to produce juveniles in significant numbers for commercial production to take place. Given these characteristics and suitable site availability with the correct environmental conditions for culture, the evolution to maturity of a specific species industry can be summarized in the following stages:

• Identification and selection of high value species together with the R&D necessary to provide an understanding of the species biology and nutrition.
• The development of reproductive technologies to close the life cycle of the species considered together with the acquisition, domestication and manipulation of broodstock to produce eggs year round.
• The industrialization of technologies required for the commercial production of juveniles and their ongrowing.
• Improvement in productivity through economies of scale and the reduction of costs through vertical integration.
• Improvement in productivity through biotechnological solutions such as genetic advancement and nutritional engineering.
• Increased market activity through commercial pressure with the development of quality labeling, processing and other value added activities.
• Streamlining of the industry due to increased production, reduced profits resulting in grouping, mergers and consolidation to remain commercially competitive.
• The maturation of the industry and the species losing its status of a high value item and becoming a commodity product.
• Species diversification in search of additional profits through a recycling of the above procedure.

TRANSFER OF TECHNOLOGY BETWEEN SPECIES

The transition from salmon to other marine species is of particular interest and importance as it involved
the transfer of on-growing technologies from Norway and Scotland to the Mediterranean for the sea bass and sea bream industries in the 1980s to 1990s. This, however, in itself was not sufficient as the reproductive technologies, zootechnical, nutritional and environmental conditions required for the culture of marine finfish juveniles were significantly different from those of salmon.

A swim up salmon fry (150 mg wet weight) is many times larger in body mass than that of a sea bream (0.35 mg wet weight), and while the salmon fry is capable at first feeding of ingesting and digesting inert particulate feeds this is not the case for many marine species. Due to the rather underdeveloped digestive system of these larvae and their behaviour, a sophisticated live food chain was required. Researchers and industry concentrated efforts to develop a species-specific nutritional strategy and to identify the correct environmental conditions enabling these very small and sensitive larvae to be successfully reared and develop sufficiently to be weaned onto inert diets before traditional on-growing technologies could then be used.

Since the 1980s, larval rearing technologies have been continually developing for the sea bass, sea bream and turbot industries and today they are well understood. Survival rates for these species have increased from less than 1% to between 20 and 40% in 10 years. In the last few years these warm water larval rearing technologies have been adapted and introduced to Norway and Scotland, enabling the mass production of a marine cold water species, the cod (*Gadus morhua*). While production of cod is expected to reach only 12,000 tonnes in 2005, this species, which occupies the same environmental conditions as salmon, offers an alternative or addition to the troubled salmon industry.

Economically, the present viability of these two species in the UK can be summarized as shown in Table 2 (R. Prickett, personal communication); and it should be noted that as technology and food conversion rates improve for cod, so should profits. History indicates however that as volume increases so market prices will reduce.

It is developments such as these that offer Europe the potential to continue expanding and diversifying its existing aquaculture industry by looking forward to new and more profitable species through the adaptation and development of existing biotechnologies and management strategies.

<table>
<thead>
<tr>
<th></th>
<th>Salmon</th>
<th>Cod</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost per kg (round)</td>
<td>€2.04</td>
<td>€2.82</td>
</tr>
<tr>
<td>Cost of harvesting/gutting etc</td>
<td>€0.423</td>
<td>€0.423</td>
</tr>
<tr>
<td>Yield (head on gutted)</td>
<td>90%</td>
<td>86%</td>
</tr>
<tr>
<td>Cost per kg (head on gutted)</td>
<td>€2.735</td>
<td>€3.765</td>
</tr>
<tr>
<td>Market price (head on gutted)</td>
<td>€2.735</td>
<td>€4.935</td>
</tr>
<tr>
<td>Profit/kg</td>
<td>0</td>
<td>€1.170 (31%)</td>
</tr>
<tr>
<td>Cost of filleting</td>
<td>€0.141</td>
<td>€0.141</td>
</tr>
<tr>
<td>Yield after filleting (head on gutted)</td>
<td>68%</td>
<td>55%</td>
</tr>
<tr>
<td>Cost/kg (fillet/skin on)</td>
<td>€4.23</td>
<td>€9.23</td>
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</table>

### Reproductive technologies

It has been estimated that less than 3% of the total world aquaculture production is based on genetically improved stocks. Norway started to work on the selection of salmon in the 1960s and it was found that there was a large genetic variability for important production traits between wild and culture stocks offering potential for improvement. The best strains were then used as a base population for a national genetic improvement programme. This has lead to the development of salmon strains that show a genetic gain of around 100% for growth rate as well as other important characteristics such as late maturing individuals, resulting in improved production characteristics and reduced production costs. A similar breeding programme carried out using tilapia in the Philippines has shown more than a 100% improvement in growth rate over 10 generations.

In both species, selection for high growth rate can result in a gain of over 10% per generation. Only in the last 10 years has European Union research focused on broodstock genetics and selection programmes for the sea bass and sea bream industry in the Mediterranean. Prior to this production was still based on wild broodstock and selection from F1 cage production, with little monitoring to avoid inbreeding.

Traditionally, preventing inbreeding has been the largest problem in fish breeding programmes, due to large full sibling groups and the lack of individual identification methods. Today the identification of individual broodstock fish using microchip tagging is commonplace. The cost of microsatellite DNA and AFLPs (amplified fragment length polymorphisms) labelling has significantly decreased enabling the tracking of pedigrees and providing linkage maps to identify quantitative trait loci, such as growth and

Table 2. Comparative profitability of salmon and cod in the UK in 2003.
disease resistance, that have commercial importance (Agresti et al., 2000). These technologies have enabled individual producers to carry out selection methods and apply them in a practical manner in the industrial environment.

Growth rate is generally the first characteristic of importance for fish farmers as it is relatively easy to select and quantify. Other traits thought to be genetically dependent, for example disease resistance and flesh quality (muscular lipid content, fat deposition), are difficult to measure and require a more complex approach often beyond the abilities of the individual farmer. Various thematic R&D programmes within the European Union are now addressing many of these issues.

Many species still prove difficult to spawn in captivity, particularly in intensive production systems, an example being sole (Solea senegalensis). Triggers for male and female maturation and ovulation are still not well understood for this and other species such as the groupers and some tuna species. Endocrine regulation of reproduction has been effectively applied in some species and hormonal implants are readily available (Zohar and Mylonas, 2001). More investigation of the environmental and nutritional requirements of many species is required as the production of viable eggs is a prerequisite to the culture of any species.

Egg quality has a significant impact on the viability, survival and growth of marine larvae. The enrichment of broodstock diets with essential fatty acids and other vitamins and minerals have been shown to relate directly with the levels of these substances in marine eggs and larvae (Watanabe, 1993; Cedra et al., 1994; Harel et al., 1994). The use of algae in the larval rearing tanks, the ‘green water technique’, is not limited to the purely nutritional side of rotifer enrichment but has other practical applications:

- Algae can act as an antibacterial agent (Austin and Day, 1990; Cooper et al., 1983). In addition, specific polysaccharides in the algae cell wall are thought to stimulate a non-specific immune response in young larvae.
- Algae have been reported to act as an in situ biological filter removing potentially harmful metabolites from the water by stripping off nitrogenous substances. They also produce oxygen through photosynthesis.
- Algae act as a light filter and diffuser facilitating an even distribution of live food and larvae within the tank system.
- Algae act as a promoter and background for the location of prey organisms, hence playing a particularly important role in the critical first feeding stage of larvae.
- Algae have been shown to stimulate the enzymatic synthesis and onset of feeding in young larvae.

In recent years photo-bioreactor systems have provided an efficient alternative to traditional sack culture systems for the production of unicellular algae. These are labour saving, automated and cost effective. Productivity using these systems can be up to 10 times greater than that achievable with traditional culture methods. Undoubtedly the success of such systems is dependent upon light availability; and the Mediterranean climate is particularly suitable.

The search for additional algal species continues, and isolates of local species are being investigated in

<table>
<thead>
<tr>
<th>Table 3. Commonly cultured unicellular planktonic algae.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillariophyceae</td>
</tr>
<tr>
<td>(Diatoms)</td>
</tr>
<tr>
<td>Chlorophyceae – green algae</td>
</tr>
<tr>
<td>Chlorella spp.</td>
</tr>
<tr>
<td>Chrysophyceae</td>
</tr>
<tr>
<td>Chlorella spp.</td>
</tr>
<tr>
<td>Eustigmatophyceae</td>
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<tr>
<td>Chlorella spp.</td>
</tr>
<tr>
<td>Haptophyceae</td>
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<tr>
<td>Chlorella spp.</td>
</tr>
<tr>
<td>Pavlova lutheri</td>
</tr>
</tbody>
</table>
Norway in an attempt to replace some of the traditional non-indigenous species. In addition, commercial companies are marketing concentrated algal pastes, delivered either alive with a limited shelf life or cryopreserved, which offer a back-up and an alternative to traditional algal production.

The second link of the live food chain is the rotifer. The duration and quantity of rotifers required varies with the species; cod, for example require up to four times the number of rotifers per animal produced when compared to the sea bream. Some species such as the sea bass may avoid this stage altogether first feeding directly on Artemia nauplii, the last link in this chain.

Rotifer production methodologies have improved over the years from an algae and yeast-based diet giving poor productivity and unpredictable results to improved culture diets. The new generation of diets enables culture densities of 2000 rotifers per ml or more to be achieved over a 4-day batch cycle. Further developments have resulted in additional improvements. For example, 5000 rotifers per ml can be achieved using concentrated fresh water chlorella algae and automatic dosing pumps; and recirculation systems using protein skimmers, in association with novel filters enable rotifer cultures to reach and be maintained at densities over 5000 per ml for prolonged periods of time. These developments have been shown to both provide significant economic benefit and importantly improve the microflora of the culture by reducing the incidence of Vibrio spp. (Suantika et al., 2003).

Ongoing European Union projects have revealed that there is considerable genetic diversity in the rotifer populations in European hatcheries showing considerable difference in performance. It is not yet clear whether it will be necessary to work with selected genotypes cultured over a limited number of generations or how these cultures will be susceptible to changing culture conditions (Sorgeloos, 2004).

A better understanding of the nutritional requirements of the fish species cultured has led to the development of a number of commercially available cultivation and emulsion type enrichment diets for both rotifers and artemia. Dietary research has indicated the importance of the (n-3) highly unsaturated fatty acids, mainly eicosapentaenoic acid (20:5(n-3), EPA), docosahexaenoic acid (22:6(n-3), DHA) and more recently the long chain n-6 PUFA arachidonic acid (20:4(n-6), ARA) has been implicated as an essential fatty acid for a variety of developing marine species (Estevez et al., 1999). It is the bioencapsulation of these (Figure 4) and other essential nutrients through the live feed chain in the ratios required by the species concerned that has led to the alleviation of several problems such as pigmentation and some deformity issues in larval rearing as well as improving survival.

These enrichment products today are available together with products that for Artemia are capable of altering the microbiological characteristics of the hatching and enrichment environments by reducing Vibrio levels to 30% or less of non-treated environments. This development both provides the farmer with custom nutritional packages and helps to reduce the possibility of disease that may be introduced through the live food chain.

### Larval nutrition and feed technologies

Larval rearing technologies are today highly intensive with up to 250 larvae stocked per litre. Until recently
the live food chain was entirely responsible for the nutrition of these larvae until weaning commenced at approximately 30 days post hatch. The role of the live food chain is still vitally important for many species, but the development of a new generation of sophisticated inert co-feeding and replacement diets have enabled the further intensification of the larval rearing process, and considerably reduced reliance upon the live food chain thereby simplifying production methodologies.

Nutrition, health and performance are intimately linked and the industry is placing increasing importance and effort on optimizing formulations and improving ingredient quality including the sourcing of fresh raw materials. A cold extrusion spherizer agglomeration system has been used to produce diets aimed at the complete replacement of the live food chain and they focus on high digestibility. Skretting use the above technology together with a patented phospholipid content of 12%. This has been reported to play an important role in the reduction of juvenile deformation and improved growth performance.

It is hoped that further developments of micronised replacement diets will both simplify and standardize future marine fish larval rearing and enable a greater number of species to be commercialized. Possibly given the restriction of the very small mouth sizes of some marine larvae, such as the groupers, and the difficulty in maintaining extremely small strains of rotifers for first feeding, highly digestible diets of this type may provide an alternative strategy for the industry.

On-growing technologies

European aquaculture is focused on the intensive rearing of fish. Salmon, some trout, sea bass, sea bream and other various round marine fish are grown in floating cage structures. Protected coastline offering abundant site availability in Norway and Scotland led to the development of the cage farming industry. Following the early success and profits made in these areas attention turned to the Mediterranean, in particular the Adriatic, Ionian and Aegean seas where similar coastlines existed. This led to the development of the sea bass and sea bream industry. As these industries developed and juvenile availability increased for each species, the type of these cage structures developed. Initially small wooden structures evolved to larger steel ones with the culture volumes increasing from 125 m³ to 3000 m³.

The need to find additional on-growing sites for expansion and to move away from conflicts with tourism and environmental concerns, particularly in the Mediterranean region, led to the rapid migration of the industry to more off-shore locations. These more remote sites had the advantage of being in deeper water, with better water exchange providing improved growth and hygiene conditions. The structures generally employed were circular polyethylene cages and these were more flexible and better suited to the harsh environmental conditions encountered. In addition, they proved to be less expensive and provided further economies of scale with volumes of 10,000 m³ or more.

These large cages are today fully automated with computerized air blown feed delivery systems operated from a centrally moored feed barge (Figure 5). Underwater monitoring and surveillance systems enable operators to observe feeding behaviour, fish health status and adjust feed delivery to ensure optimal growth and feed conversion ratios (FCR).

These systems have been found to be suitable for most European weather conditions, however some fully exposed sites are experimenting with submersible cage systems to avoid extreme conditions. These new technologies are primarily being investigated in other areas of the world where tropical storms and the lack of protected coastlines make traditional cage systems impractical.

The future of cage on-growing technologies will continue with the establishment of more offshore cage systems and perhaps even larger structures will be developed to accommodate the requirements of new species such as the tunas.

Land-based on-growing technologies in Europe can be split into open flow through raceway-type systems, commonly used for the production of trout, and re-circulation systems for eels, tilapia and turbot amongst others. The flow-through systems may exploit the benefits of waste warm water from power stations where a number of species are cultured including sole, sea bass and sea breams. Semi-intensive pond culture of marine and brackish water species also takes place in Spain and Italy and the pond culture of carps exists in many countries of Europe. Highly intensive land based systems are used for the pre-on-growing of juvenile sea bass and sea bream in Spain where the rigours of off-shore on-growing systems dictates that the size of the juveniles recruited should be in the order of 20 g. Typically these systems employ liquid oxygen and use where possible borehole water, which has proved to have the benefits of
constant temperatures, low bacterial levels and high water quality without the need for expensive pre-treatment.

Land-based systems are the only effective method of producing some flat fish species; and while in Europe land prices are at a premium, the value and market potential for some species (sole $11/\text{kg, whole}$) is such that significant investment is already underway. The potential for the development of vertically stacked shallow raceway systems controlled by re-circulation technologies is therefore of interest.

**On-growing feed technologies**

Modern European aquaculture feed production developed in parallel with the needs of the salmon and trout industries. Licensing restrictions based upon feed consumption and waste product release into the environment together with the commercial pressures of feed usage and improved growth rates have resulted in more efficient and cost effective formulations.

High energy extruded feeds used in the salmon industry today can contain fat levels of over 30% and feed conversion rates have dropped to below 1:1. The efficiency of these diets has been cross-checked by *in vivo* testing using mink as a target animal to determine among other things protein digestibility.

Excess production capacity in northern European feed mills was quickly adapted to meet the demand of the developing sea bass and sea bream industry in the Mediterranean. Initial pelletized feeds were replaced with extruded diets and formulations adapted to meet the demands of individual species were developed as production grew.

Consumer confidence and food safety issues are important factors in aquaculture. Following the BSE outbreaks, the European Union banned the inclusion of ingredients derived from terrestrial animal by-products. This has stopped the incorporation of hemoglobin, blood meals, meat, bone and feather meals amongst other ingredients. In addition to this, many sales outlets and the large supermarket chains require European feeds to be certified GMO-free as part of their drive to satisfy consumer demand and perception.

The newly established European Food Safety Authority (EFSA) runs risk analysis and risk management and promotes an integrated approach to the responsibility of feed manufacturers, farmers and food operators on the traceability of feeds, food and their ingredients.

These actions, while essential to regulate the industry and address consumer and public health issues, severely restrict the formulations available for aquaculture. This in turn places a heavy load on the limited resource of fish meal as a protein source and adds to the cost of feed at a time when the industry is striving to reduce production costs by all means possible. Fish meal and oil substitutes are emerging...
now as viable, economic partial alternatives. The inclusion of digestibility enhancers and organic chelated trace minerals provides better bioavailability of important nutrients.

Feed quality may also be affected by poor storage conditions or raw material quality. This can result in the development of mycotoxins that can increase disease sensibility, result in lower growth and poor FCRs (Lovell et al., 1994). Modern natural glucomannan mycotoxin adsorbents are capable of absorbing a broad spectrum of mycotoxins directly from the digestive tract preventing their absorption and subsequent bioavailability.

**Health and disease issues**

Parasitic, bacterial and viral diseases cause considerable financial loss to the European industry. Disease problems in the marine sector originate from a diverse range of infectious agents, which have been reviewed by Le Breton (1996) and Rodgers and Furones (1998), a list that has rapidly developed and is continually expanding.

The development of molecular techniques for the identification and screening of pathogens offers the potential to improve disease prevention and control. The sensitivity of nucleic acid probes now enables the detection of subclinical carriers of some infections; and this is an important development and tool for the establishment of specific pathogen-free broodstocks.

Hatchery production units try to avoid the introduction of opportunistic pathogens through treatment of the incoming water supplies using a variety of filtration methods. Sterilization, either by UV and (or) ozone, is common practice in both freshwater and marine environments. Production scheduling now includes specific periods where either units of, or the whole hatchery are shut down, cleaned and sterilized and all-in-all-out batch production provides regular sanitary control. Areas within the hatchery environment are kept as discrete as possible with the minimum of interaction by working personnel and equipment from one area to another. Isolated quarantine facilities are employed to prevent the introduction of disease.

In larval rearing various strategies have been proposed for controlling the microflora of this environment. Mature water and the addition of probiotics either through the live food chain or directly in the larval rearing tank have been used. The larval pre-feeding and first feeding stages are critical to the establishment of the microflora of the gut. (Bergh et al., 1994; Munro et al., 1993) and the presence of opportunistic pathogens at this stage has been shown to lead to disease (Grisez et al., 1996).

Fish transfer from the hatchery to the pre-on-growing or on-growing facilities necessitates the transfer of fish from a protected to an unprotected environment in which they might come into contact with a variety of different pathogens. It is possible to protect against certain diseases with vaccines, and a limited number are available for commercial use. Vaccination takes places prior to the transfer from the hatchery facilities to the on-growing units, but due to the limited duration of protection offered to fish further vaccination may be necessary during the on-growing cycle.

With the complexity of vaccine licensing and the restricted use of antibiotics and some other therapeutic agents the industry is turning to other methods of prophylaxis and control to improve the fish health status. The concept of nutritional supplementation, the use and blending of selected nutrients, immunostimulants and immunomodulators are urgently being considered by the aquaculture industry as it learns of their effectiveness in terrestrial animal culture.

**Conclusions**

European aquaculture is expected to show growth in the marine sector and the success of individual operations will depend on the successful application of a variety of multidisciplinary activities. Economic viability must be linked to better marketing strategies and food safety. Transparency, traceability, quality and sustainability issues are at the forefront of European concerns and actions. Technological improvements are expected to continue to improve cost efficiency and stimulate further species diversification at a time when fisheries production is stagnant and in certain sectors in decline. Simplified legislation and licensing procedures have been called for and continued and coherent policies for research and development are essential.

**References**


Fish meal and fish oil use in aquaculture: global overview and prospects for substitution

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Introduction

Although aquaculture’s contribution toward total world fisheries landings has increased six-fold over the past three decades, increasing from 5.3% to 34.1% from 1970 to 2001 (Figure 1), the finfish and crustacean aquaculture sector is still highly dependent upon marine capture fisheries for sourcing key dietary nutrient inputs such as fish meal and fish oil. In fact when viewed in wet fish weight equivalents, although only about 17.7 million metric tons (mmt) or 37% of total global aquaculture production in 2001 (Figure 2) were finfish and crustacean species whose production was dependent upon the use of compound aquafeeds, these species consumed the equivalent of 17 to 21 mmt of marine pelagics on a wet weight basis.

This paper reviews the current and predicted global use and demand for fish meal and fish oil with compound aquafeeds for farmed finfish and crustaceans, including prospects for substitution; aquafeeds including commercially compounded diets, farm-made aquafeeds, and/or whole marine food/feed organisms as well as fresh/frozen fish, molluscs and crustaceans. Particular emphasis is placed on the urgent need for the aquafeed-fed finfish and crustacean aquaculture sector to reduce its current dependence upon potentially food-grade marine capture fishery resources for sourcing its major dietary protein and lipid nutrient inputs (in the form of fish meal and fish oil), and to seek alternative more sustainable feed resources; the long term

Figure 1. Contribution of aquaculture to total world fisheries landings 1970-2001.
evaluation and use of single cell proteins (SCP) being particularly encouraged and holding much promise.

**Current fish meal and fish oil usage**

As mentioned previously, the finfish and crustacean aquaculture sector is currently heavily dependent upon capture fisheries for sourcing key nutrients and feed ingredients for compound aquafeeds. These include high quality animal proteins and feeding attractants (fish meal, and to a lesser extent fish solubles, shrimp meal, squid meal, fish/squid liver meals, fish/crustacean hydrolysates, and krill meal) and essential dietary lipids (fish oil, and to a lesser extent fish and squid liver oils); Hardy and Tacon, 2002; New and Wijkström, 2002; Tacon, 2003a). This dependency upon fish meal and fish oil is particularly strong for those higher value species feeding high on the aquatic food chain, including all carnivorous (i.e. fish/invertebrate animal-eating) finfish species and most omnivorous/scavenging crustacean species.

Finfish and crustacean species that are currently reliant upon fish meal as the main source of dietary protein in compound aquafeeds include: finfish - all farmed marine finfish (excluding mullets and rabbitfish); diadromous species - salmonids (salmon, trout, char), eels, barramundi, sturgeon; freshwater species - mandarin fish, pike, pike-perch, snakehead, certain freshwater Clarias catfishes; and crustaceans - all marine shrimp, crabs, and freshwater prawns. A similar dependency also exists for fish oil (as the main source of dietary lipids and essential fatty acids in compound aquafeeds) for the above species, with crustaceans currently being less dependent than carnivorous finfish due to the lower levels of dietary lipids generally used in crustacean feeds. In addition to the above, fish meal and fish oil are also commonly used as a secondary source of dietary protein (usually included at low dietary inclusion levels) and lipid for many omnivorous cultured finfish species, including freshwater carps, tilapia and catfish (Tacon, 2003b).

Table 1 shows the estimated global use and demand for fish meal and fish oil within compound aquafeeds according to the author, with estimates from the International Fish Meal and Fish Oil Manufacturers Association (IFFO; Pike and Barlow, 2003) given in italics. From these data it can be seen that the total production of industrially compounded aquafeed production in 2001 was about 16.7 mmt (Figure 3), with aquafeed production currently representing only about 3% of total global industrial animal feed production (estimated at 612 mmt in 2003; Figure 4).

The major species groups dependent upon compound aquafeeds in 2001 include the non-filter feeding carps (8.0 mmt of aquafeeds used in 2001), marine shrimp (2.1 mmt), salmon (1.56 mmt), marine finfish (excludes mullets; 1.21 mmt), tilapia (1.16 mmt), trout (0.74 mmt), catfish (0.60 mmt), freshwater crustaceans (0.52 mmt), milkfish (0.42 mmt) and eels (0.37 mmt; Figure 3).
Table 1. Estimated global use and demand for fish meal and fish oil within compound aquafeeds (2001 species group aquaculture production estimates taken from FAO, 2003)

<table>
<thead>
<tr>
<th>Species group</th>
<th>2001</th>
<th>2002</th>
<th>2005</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MARINE SHRIMP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>-</td>
<td>1,387</td>
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<td>-</td>
<td>1,353</td>
<td>-</td>
<td>1,999</td>
</tr>
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<td>Predicted growth, APR, %/year</td>
<td>5</td>
<td>-</td>
<td>5</td>
<td>-</td>
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<tr>
<td>IFFO estimate, %/year</td>
<td>-</td>
<td>5</td>
<td>5</td>
<td>-</td>
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<tr>
<td>Percent on feeds, %</td>
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<td>-</td>
<td>87</td>
<td>92</td>
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<td>-</td>
<td>90</td>
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<td>1.6</td>
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<td>-</td>
<td>1.6</td>
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<tr>
<td>Total aquafeeds used, tmt</td>
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<td>-</td>
<td>14</td>
<td>4</td>
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<td>IFFO estimate, %</td>
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<td>Average fish oil content, %</td>
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<td>-</td>
<td>1</td>
<td>1</td>
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<td>-</td>
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<td>26</td>
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<td>IFFO estimate, tmt</td>
<td>-</td>
<td>39</td>
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<tr>
<td><strong>FRESHWATER CRUSTACEANS</strong></td>
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<td>Total production, tmt</td>
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<td>-</td>
<td>809</td>
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<td>Growth, APR, %/year</td>
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<td>8</td>
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<td><strong>MARINE FISH</strong></td>
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<td>80</td>
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<td>1.8</td>
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<td>-</td>
<td>32</td>
<td>24</td>
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<td>6</td>
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**EEL**

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<tr>
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<td>2</td>
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**MILKFISH**

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<td>-</td>
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<td>1.6</td>
<td></td>
</tr>
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<tr>
<td>*IFFO estimate, %</td>
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<tr>
<td>Average fish oil content, %</td>
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<td>*IFFO estimate, %</td>
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<td>*IFFO estimate, tmt</td>
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<td>-</td>
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**FEEDING CARP**

| Total production, tmt*             | 10,549 | 13,947 | 18,664 |
| *IFFO estimate, tmt                | -      | 15,584 | -      |
| Growth, APR, %/year                | 7      | 6     | -     |
| *IFFO estimate, %/year*            | -      | 7     | -     |
| Percent on feeds, %                | 38    | 42    | 50    |
| *IFFO estimate, %                  | -      | 30    | -     |
| Species economic FCR               | 2.0    | 1.8   | 1.6   |
| *IFFO estimate, %                  | -      | 1.8   | -     |
| Total aquafeeds used, tmt          | 8,017  | 10,544 | 14,931 |
| *IFFO estimate, tmt                | -      | 8,415 | -     |
| Average fish meal content, %       | 5     | 3     | 0     |
| *IFFO estimate, %                  | -      | 4     | -     |
| Average fish oil content, %        | 0     | 0     | 0     |
| *IFFO estimate, %                  | -      | 0     | -     |
| Total fish meal used, tmt          | 401    | 316   | 0     |
| *IFFO estimate, tmt                | -      | 337   | -     |
| Total fish oil used, tmt           | 0      | 0     | 0     |
| *IFFO estimate, tmt                | -      | 0     | 100   |

**TILAPIA**

| Total production, tmt              | 1,385  | 1,694  | 2,376  |
| *IFFO estimate, tmt                | -      | 1,449  | -      |
| Growth, APR, %/year                | 6      | 7     | -     |
| *IFFO estimate, %/year*            | -      | 7     | -     |
| Percent on feeds, %                | 42    | 50    | 60    |
| *IFFO estimate, %                  | -      | 40    | -     |
| Species economic FCR               | 2      | 1.8   | 1.6   |
| *IFFO estimate, %                  | -      | 1.8   | -     |
| Total aquafeeds used, tmt          | 1,163  | 1,525  | 2,281  |
| *IFFO estimate, tmt                | -      | 1,043  | -      |
| Average fish meal content, %       | 6      | 3     | 0     |
| *IFFO estimate, %                  | -      | 7     | -     |
| Average fish oil content, %        | 1      | 0.5   | 0     |
| *IFFO estimate, %                  | -      | 1     | -     |
| Total fish meal used, tmt          | 70     | 46    | 0     |
| *IFFO estimate, tmt                | -      | 73    | -     |
| Total fish oil used, tmt           | 11.6   | 7.6   | 0     |
| *IFFO estimate, tmt                | -      | 10    | 11    |

**CATFISH**

| Total production, tmt              | 434    | 489   | 567   |
Table 1. Continued

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<th>2005</th>
<th>2010</th>
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<td>3</td>
<td>-</td>
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<td>94</td>
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<td>-</td>
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<td>1.4</td>
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</table>

CARNIVOROUS FRESHWATER FISH

| Total production, tmt | 551 | - | 946 |
| Growth, APR, %/year | - | 7 | - | 7 |
| Percent on feeds, % | - | 20 | - | 60 |
| Species economic FCR | - | 2.4 | - | 2.0 |
| Total aquafeeds used, tmt | - | 264 | - | 1,135 |
| Average fish meal content, % | - | 15 | - | 10 |
| Average fish oil content, % | - | 6 | - | 7 |
| Total fish meal used, tmt | - | 40 | - | 114 |
| Total fish oil used, tmt | - | 16 | - | 79 |

TOTAL GLOBAL ESTIMATES

| Total fed production, tmt | 17,690 | - | 22,836 | 30,472 |
| IFFO estimate, tmt | - | 22,321 | - | 37,011 |
| Total aquafeed production, tmt | 16,700 | - | 21,111 | 28,596 |
| IFFO estimate, tmt | - | 15,794 | - | 32,378 |
| Total estimated fish meal used, tmt | 2,614 | - | 2,294 | 1,550 |
| IFFO estimate, tmt | - | 2,127 | - | 2,854 |
| Total estimated fish oil used, tmt | 594 | - | 478 | 447 |
| IFFO estimate, tmt | - | 732 | - | 953 |
| Total fish meal + fish oil used, tmt | 3,208 | - | 2,772 | 1,997 |
| IFFO calculated this paper, tmt | - | 2,949 | - | 3,807 |
| Equivalent pelagics used, tmt | 16,040 | - | 13,860 | 9,985 |

1 Total reported farmed species group production for 2001 (FAO, 2003), and estimates for 2005 and 2010 (Tacon, 2003b)
2 International Fish meal and Fish Oil Organization (IFFO) estimated farmed marine shrimp + crab production for 2002 and estimates for 2005 and 2010 (Pike and Barlow, 2003)
3 Estimated Annual Percent Rate of Growth of farmed species group production 2001/2002 to 2010 (APR, %)
4 Estimated percent of total species group production on aquafeeds
5 Estimated average species group economic food conversion ratio (total food fed / total species group biomass increase)
6 Estimated total species group aquafeed used (total species group production x FCR)
7 Freshwater crustaceans includes freshwater prawn, river crab and crayfish
8 Marine finfish species group excludes mullets
9 Feeding carp species excludes filter feeders such as silver carp, big head carp and catla
10 Includes Chinese bream, mandarin fish, yellow croakere, long-nose catfish (carnivorous/omnivorous) but excludes eel (Pike and Barlow, 2003)
11 Excludes filter feeding fish species (5,878 tmt in 2001), freshwater fish species (species unknown: 2,259 tmt in 2001), marine crabs and other marine crustaceans (200 tmt), Mandarin fish (116 tmt in 2001), and other miscellaneous freshwater fish species (including climbing perch, snakeheads, colossoma, gourami ca. 165 tmt in 2001).
12 Using a mean fish meal+fish oil to pelagics conversion ratio of 1:5 (FAO conversion factor for fish meal)

Concerning fish meal and fish oil usage, it is estimated that the compound aquafeed sector consumed about 2.62 mmt of fish meal and 0.59 mmt of fish oil in 2001 (Table 1), or equivalent to 43.1% and 53.6% of the total global production of fish meal (6.08 mmt) and fish oil (1.10 mmt), respectively (FAO, 2003).

On a species group level salmonids consumed the largest proportion of fish meal and fish oil in 2001 (29.4% and 64.5% of totals used in aquafeeds, respectively), followed by marine fish (22.6% and 20.3%), marine shrimp (19.3% and 7.0%), feeding carp (15.3% in the case of fish meal) and eels (6.9%
The total use of fish meal and fish oil in compound aquafeeds is almost certainly higher than the figure given above, as an additional 2.6 mmt of finfish and crustacean production (equivalent to 10% total finfish and crustacean production) was not included in these calculations (includes unknown freshwater fish species (2.26 mmt in 2001), marine crabs and other marine crustaceans (0.2 mmt), Mandarin fish (0.12 mmt), and other miscellaneous freshwater fish species). According to IFFO (Pike and Barlow, 2003) fish meal and fish oil usage within compound aquafeeds in 2002 was estimated to be 2.22 mmt and 0.73 mmt, respectively (Table 1).

The total estimated use of fish meal and fish oil in aquafeeds (3.2 mmt in 2001, dry basis) was equivalent to the use of 12.8 to 16.1 mmt of pelagics (using a dry meal/oil to wet fish weight equivalents conversion factor of 4 to 5) for the production of 17.69 mmt of the major farmed-fed finfish and crustacean species in 2001. Cultured species groups currently consuming more fish through feeding than is being produced through farming in 2001 included marine eels (current pelagic input per unit of production 3.4-4.2), marine fish (2.9-3.7), salmonids (2.6-3.3), marine shrimp (1.7-2.1), freshwater crustaceans (1.0-1.3), whereas, net fish producers included milkfish (0.33-0.42), catfish (0.28-0.35), tilapia (0.24-0.29), and feeding carp (0.15-0.19).

Moreover, coupled with the use of trash fish as a direct food source for farmed fish and crustaceans in some Asian countries (China reportedly using 4 mmt of trash fish as feed for marine finfish and crustaceans in 2000; D’Abramo et al., 2002), it is estimated that the aquaculture sector consumed the equivalent of 17-20 mmt of fish as feed in 2001 (either in the form of fish meal, fish oil or trash fish, expressed in live weight equivalents) for the total production of 17.69 mmt of aquafeed-based farmed fish and crustaceans in 2001. However, in contrast to the 8 to 11% annual growth rate of the aquaculture sector over the past decade, the proportion of the global fish catch destined for non-food uses (including for reduction into fish meal and fish oil, or for direct animal

Figure 3. Estimated global compound aquafeed production in 2001 for major farmed species (values expressed as % total aquafeed production, dry as-fed basis).

Figure 4. Estimated global industrial animal feed production in 2001 for major farmed species (values expressed as % dry as-fed basis).
feeding) has remained relatively constant, in recent years fluctuating from a low of 25.3 mmt in 1998 (strong El Niño year) to a high of 34.8 mmt in 2000 (Figure 7); total capture fisheries in 2001 reported as 92.4 mmt, including 61.1 mmt destined for direct human consumption and 31.3 mmt or 33.9% destined for non-food uses (FAO, 2003).

Future fish meal and fish oil usage

It follows from the above discussion that for those aquaculture species and exporting/importing countries currently dependent upon the use of these relatively finite fishery commodities as feed inputs, consumption of these commodities will have to

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**Figure 5.** Estimated global use of fishmeal in compound aquafeeds in 2001 by major cultivated species (expressed as % total fish meal used within aquafeeds, dry as-fed basis).

**Figure 6.** Estimated global use of fish oil in compound aquafeeds in 2001 by major cultivated species (expressed as % total fish meal used within aquafeeds, dry as-fed basis).

**Figure 7.** World finfish and shellfish production from capture fisheries and aquaculture, and disposition of the catch 1970-2001 (expressed in million metric tons, live weight equivalents; FAO, 2003).
increase if current dietary inclusion levels are to be maintained. For example, according to IFBO (Barlow and Pike, 2003) the aquaculture sector’s consumption of fish meal and fish oil is expected to increase from 34% (2,217 tmt) and 56% (732 tmt) of the total global production of fish meal and fish oil in 2002, to 48% (2,854 tmt) and 79% (953 tmt) in 2010, respectively (Table 1); this increase being equivalent to a 29-30% increase in global fish meal and fish oil usage by the aquaculture sector from 2002 to 2010.

The above predictions by IFBO differ from those of the present author and others (Hardy and Tacon, 2002; Tacon and Forster, 2000), who estimated that fish meal and fish oil use by the aquaculture sector will actually decrease rather than increase in the long term (Table 1). Thus, from the data presented in Table 1 it is expected that usage will decrease by 40% in the case of fish meal (from 2,614 tmt in 2001 to 1,550 tmt in 2010) and 25% in the case of fish oil (from 594 tmt in 2001 to 447 tmt in 2010, Table 1).

The main reasons why fish meal and fish oil use by the aquaculture sector is expected to decrease in the long term is due to a combination of increasing economic/market pressures placed upon the fish meal and fish oil manufacturing industry and animal feed compounder on the one hand; and the consequent search, development and use of lower cost and more sustainable alternatives by the aquafeed manufacturing sector on the other hand so as to maintain profitability and sustain the growth of the feed-dependent aquaculture sector. Examples of increasing economic/market pressures placed upon the fish meal/aquafeed manufacturing sector include:

- The increasing market demand for the production and use of less environmentally contaminated fish meals and oils (through the selection of less contaminated fish stocks and/or through increasing legislative controls limiting fish meal/fish oil use in aquafeeds; Hites et al., 2004; Jacobs et al., 2002; Pike, 2002; Smith et al., 2002).
- The increasing global demand for the use of potentially food-grade pelagics (including mackerel, sardines, herring, pilchards, anchovies) for direct human consumption rather than for reduction into fish meal and fish oil (Wray, 2001),
- Increased global competition for available stocks of fish meal and fish oil by the rapidly emerging aquafeeds and compound animal feed manufacturing sector in developing countries (including China, Thailand, Indonesia, India, Chile, Brazil) D’Abramo et al., 2002; FAO, 2003; Tacon, 2003a; Figure 4),

Moreover, as a result of increased aquaculture production and decreasing fish/shrimp market prices (Harvey, 2003; Hinrichsen, 2003), nutritionists and feed manufacturers alike have been forced to reduce feed costs (through the development of fish meal and fish oil replacers) and/or by improving on-farm feed performance so as to maintain profitability.

Sustaining aquaculture production: substitution prospects

Clearly, in view of the fact that wild fish stocks have remained relatively static over the last decade (Figure 7), the aquaculture sector has no choice but to reduce its dependence upon capture fisheries for sourcing its dietary protein and lipid nutrient inputs if it is to sustain its annual growth rate of 8.8% per year since 1970 (Figure 1). Although this will be a relatively simple task for omnivorous/herbivorous finfish and crustacean species (in view of their more flexible feeding habits/food preferences and available feed resources), this will be more difficult for carnivorous species.

To date the majority of recent effort has focused on the development and use of the following major fish meal replacers:

Terrestrial vegetable proteins (TVP), includes protein-rich oilseed and grain by-product meals including soybean, rapeseed, corn gluten, wheat gluten, and to a lesser extent pea and lupin meals (Abdelghany, 2003; Abery et al., 2002; Barros et al., 2002; Bautista-Teruel et al., 2003a, 2003b; Borlongan et al., 2003; Cheng and Hardy, 2002a; Cheng et al., 2003; Cremer et al., 2003; Du and Niu,
Fish meal and fish oil use in aquaculture: global overview and prospects for substitution

2003; Farhangi and Carter, 2001; Francis et al., 2001; Glencross et al., 2003a, 2003b, 2003d; Grisdale-Helland et al., 2002; Hari and Kurup, 2003; Jahan et al., 2003; Lee et al., 2002; Maina et al., 2003; Mente et al., 2003; Opstvedt et al., 2003a, 2003b; Penaflorida, 2002; Pereira and Oliva-Teles, 2002; Peres et al., 2003; Refstie and Tiekstra, 2003; Richter et al., 2003; Rinchard et al., 2002; Dabrowski et al., 2003; Siddhuraju and Becker, 2003; Singh et al., 2003; Takagi et al., 2001; Thiessen et al., 2003a, 2003b). According to the FAO agricultural statistical database the total production of plant oil cakes and meals in 2002 was over 177 mmt (Figure 8), as compared with a total global production of just over 7 mmt for fish meal and fish oil.

Terrestrial animal proteins (TAP), includes animal by-product meals: poultry by-product meal, meat meal, meat and bone meal, and to a lesser extent feather meal and blood meal (Abdel-Warith et al., 2001; Bharadwaj et al., 2002; Cheng and Hardy, 2002b; Cheng et al., 2002; Forster et al., 2003; Mendoza et al., 2001; Menasveta et al., 2003; Millamena and Golez, 2001; Millamena, 2002; Tan et al., 2003; Williams et al., 2003a, 2003b; Woodgate, 2004a, 2004b; Zhu and Yu, 2003).

Single cell proteins (SCP), includes unicellular and filamentous algae, yeasts and bacteria (Lara-Flores et al., 2003; Li and Gatlin, 2003; Nates and Tacon, 2003; Nates et al., 2002; Olvera-Novoa et al., 2003).

Of the above-mentioned feed sources, considerable further work still needs to be undertaken concerning the use and suitability of SCP as dietary fish meal replacers for both finfish and crustaceans. These products hold particular promise by virtue of their ability to be produced from renewable resources and/or agricultural/petrochemical waste streams/substrates, their rapid growth rate, high dietary protein content and nutritive value (generally devoid of anti-nutrients and overt nutrient imbalances), and the ability to tailor their nutritional composition to approximate needs of the cultured target species (through direct nutrient modifications during the fermentation or growth process).

Finally, total replacement of fish oil with commercially available plant and animal oils has been more problematic, especially within carnivorous marine and diadromous finfish species. Studies where plant and animal oils (including soybean, rapeseed, linseed oils and terrestrial animal fats) have achieved some success as fish oil replacers (depending upon

![Total production of plant oil cake and meal in 2002: 177.7 mmt](image)

Figure 8. Estimated global production of plant oilseed cakes and meals in 2002 (source: FAOSTAT Agriculture Database).
the species farmed) have included: Ballestrazzi, 2003; Bell et al., 2002, 2003a, 2003b; Bransden et al., 2003; Bureau, 2004; Grisdale-Helland et al., 2002b; Glencross et al. 2003c; Martino, 2003; Montero et al., 2003; Ng et al., 2003; Raso and Anderson, 2003; Regost et al., 2003a, 2003b; Tocher et al., 2002; Turchini et al., 2003. However, as in the case of dietary protein sources, considerable potential also exists concerning the further development and use of SCP for the production of dietary lipid supplements and ingredients rich in long chain polyunsaturated fatty acids and other essential lipid soluble nutrients, including carotenoid antioxidants, phospholipids, specific steroids etc. (Nates and Tacon, 2003b).

Clearly, the long term sustainability of the aquaculture and compound aquafeed sectors will only be ensured if ingredients can be sourced whose production can keep pace with the growth of the sector (including the competing terrestrial livestock meat production sector; Figure 9), and whose nutritional composition and characteristics are flexible and can be tailored to the growth and ever changing requirements of the sector.

References


Figure 9. Total global farmed terrestrial and aquatic meat production 1970-2001 (source: FAOSTAT, 20034).
Fish meal and fish oil use in aquaculture: global overview and prospects for substitution


Creating alternative protein sources for aquafeeds using applied enzyme technologies

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Introduction

One of the great challenges of the next decade will be to provide sufficient high quality proteins for the rapidly growing aquaculture industry. A wide range of species to be farmed will necessitate sourcing feed proteins that are able to meet the specific requirements of the target species. Fish meals have traditionally provided a large proportion of the protein required by aquaculture species, and even if there is no immediate shortage of supply, there are requirements for alternatives for a variety of reasons. One of the key aspects is the high cost of fish meals, mainly caused by an imbalance of supply and demand. Although this may vary from year to year because of supply variation, the trend toward higher prices has been steadily increasing because of growing demand.

There are bountiful supplies of many protein sources that may, in principle, be suitable for inclusion in aquafeeds. However, just because an ingredient has a high crude protein level does not mean that it is a viable alternative, and a much greater depth of knowledge must be attained before a product can be accepted. Of course over the last decade, many researchers have been considering the need for fish meal alternatives, and how they can fit into cost effective and environmentally balanced feeds (Bureau and Viana, 2003).

This paper discusses a more fundamental stepwise approach to the problem of supplying alternative protein sources using, where appropriate, applied enzyme technology. This stepwise approach will be described, followed by a case study following the same principles, using enzyme processed feather protein for aquaculture feed as an example.

Application principles for upgrading by-products

WHAT ARE THE PROBLEMS WITH BY-PRODUCTS?

Poor nutritional value. Many potential sources of protein have a low nutritional value. This may be manifest as a poor or low digestibility of the protein or amino acids, or because the amino acid profile is not suitable for the target species.

Antinutritional factors. Certain (mainly vegetable) proteins contain anti-nutritional factors; the best known of which is probably trypsin inhibitor, a digestive enzyme inhibitor present in raw processed soya beans. Many antinutritional factors can be neutralised by a process step, and in the case of trypsin inhibitor, it is inactivated by a heat processing stage.

Stigma/prejudice. Some feed ingredients have a long history of having of little or no value as an animal feed ingredient. In this situation, additional promotional work will be required if efforts to increase value in terms of enhanced nutritional value have succeeded, but stigma still exists.

Environment. Some products have an adverse environmental impact if they cannot otherwise be used, either in an unmodified or added value state. This may manifest itself (in the worse case) as disposal in a landfill, which results in both a loss of nutrients and a negative environmental impact by leaching pollutants into groundwater or by increasing biogas production.

Legislation. In some cases legislation may ban the use of certain products for use in animal feeds. In Europe, a temporary ban on certain processed animal proteins (OJEC, 2000; 2000/766/ec), has resulted in
a temporary loss of protein resource for animal feeds. It is hoped that the temporary ban will be lifted in stages during 2004/5, resulting in some key animal proteins being available for farmed animals such as aquaculture species.

Supply/demand imbalance. If the supply/demand considerations do not initiate interest in upgrading, then development of an upgrading strategy is more difficult. While the current situation of uncertain and expensive supply failing to meet growing demand is not critical, these circumstances must be addressed when developing an upgrading protocol.

No specific target species. If there are no specific target species for the by-product, then making an economic model for the upgrading process is particularly difficult. Identification of key species as the target market for the upgrading protocol can be crucial to the success of an upgrading project.

FOCUS ON TARGET SUBSTRATE

Protein and nitrogen compounds. These are the key parameters. Much of the nutritional value analysis will depend on protein, particularly if protein is a major nutrient in the overall nutrient profile of the product. Major emphasis is now placed upon the in vivo digestible amino acid profile of ingredients, so this is a vital parameter to consider.

Complex carbohydrates. Although protein is the major focus for aquaculture feeds, the presence of complex carbohydrate may limit how much positive effect can be achieved by targeting protein alone. Therefore, if complex carbohydrates are present, an enzyme step to minimise any potentially negative actions on protein digestibility might be introduced.

Fats, oils and waxes. Fats and oils may inhibit enzyme activity in some circumstances. If the natural level of fat is high or there is major contamination, then steps may be taken to reduce the potentially negative effect that the fat may have on the proposed protein upgrading step.

Minerals. Minerals present in high quantities may affect any proposed protein upgrading step by interaction with the substrate or enzyme. Thus the effect of certain minerals on both of these parameters should be understood and compensated for if possible.

Potential interactions. It is critical to know in detail the nutrient profile of the target product. To be able to move the upgrading project forward, an understanding of the sample analysis and its provenance is essential. These details will be required for both targeting the parameters to be upgraded and for defining value to the target species.

THE KEY PROCESS PARAMETERS

The parameters discussed below are all factors that require a stepwise approach to the optimisation of the process. Here the benefit of using laboratory and pilot scale development equipment cannot be overestimated. The iterative approach required is possibly achieved most successfully in applied research establishments such as the Alltech Bioscience Centres.

First the substrate must be defined. For example, the protein content and configuration of a raw material should be known before any attempt to upgrade is made. Any potential interaction with another nutrient such as complex carbohydrate that might affect protein or amino acid availability should also be considered.

In general, particle size should be as small as practical to ensure that the largest surface area possible is available for enzymic hydrolysis. If an additional pre-milling or size reduction step is required, then this requirement needs to be confirmed and included in the process development steps.

As a rule, sufficient active water content is needed to ensure optimal hydrolysis. The optimum range may be narrow or wide, and this optimisation also requires previous knowledge or new development research, possibly using a pilot plant. When considering moisture, processes that operate for all or some of the time in a high moisture environment may have a significant advantage over those that are dehydrated before any enzymic action might be possible.

It is well known that most enzymes are somewhat sensitive to pH, and this range must be considered when matching the enzyme and substrate, particularly if any pH adjustment is likely to be required.

As with pH, temperature and its control are critical. Too low a temperature may mean processing time is too long to be commercially viable. If temperature is too high, there are potential problems with enzyme inactivation when critical limits are reached. Of course many commercial enzymes have a broad range of temperature optima, so the challenge is to match the optimum enzyme temperature with the process temperature without having to make excessive alterations to the latter.

Mixing is a vital feature that may be overlooked, particularly when additional equipment is needed to
facilitate optimal enzyme hydrolysis. If mixing is inadequate, then the desired improvements may not be realised, and the project may fail. Therefore the importance and scope of any mixing needs to be considered as early in the project as possible, as additional capital may be needed if a mixing stage is needed.

The enzyme of choice will be determined by the substrate, but many of the above mentioned factors may need to be taken into account before settling on one or more enzymes to be used.

In some circumstances enzymes alone may not be completely effective and great benefit could be obtained by introducing a co-factor. For products with a complex chemical composition, the addition of a co-factor designed to achieve a specific purpose may allow the enzyme to be greatly more effective.

Stabilization is critical. It is assumed that the materials of interest are of a high or unstable moisture content after the enzyme activity is complete. The choice of stabilization method depends on a number of factors, including the possible environmental challenges of one route vs another, the cost of equipment, operating (fuel and drying) cost and finished product transport cost.

The application of heat to dry the product will both inactivate any residual enzyme activity and reduce water activity to a level where the product is stable. Wet preservation is generally achieved by the addition of acid or alkali with the purpose of inactivating the enzyme and inhibiting any possible spoilage organisms. The moisture content is unchanged from the starting level, and therefore transport of finished product includes cost of transporting water as well as the product.

COMPARING CONVENTIONAL AND ENZYME PROCESSES

In the context of processes that are suited for the application of enzyme technology, there are existing processing conditions usually described as ‘conventional’. Here, benchmarks for a variety of process and product parameters are already known, and these can be used to evaluate the differences and improvements that enzyme technology can bring. In most cases a monetary value change can be ascribed to the differences seen, although this is not always possible with concepts such as product prejudice.

There are a number of key benchmarks that can be considered when comparing conventional and enzyme processing. Energy requirements are one such consideration. Many processes using enzyme technology will require less energy input in a variety of forms e.g. mechanical or electrical power, and steam generated to provide heat for processing and dehydration. Likewise, environmental impact of conventional and enzyme processes differs. Emissions to the environment in the form of solids, gases, or liquids must all be controlled and in the future minimised. The process and product that can deliver environmental benefits over a conventional method will be more valuable in the long term.

A product nutrient comparison of conventional and enzyme-processed products may not reveal major or dramatic differences using the proximate analysis. In reality, much of the value added by enzyme processing must be considered using other evaluation techniques such as in vivo response or animal performance. In vivo response is the penultimate evaluation method, but may involve both costly and time-consuming techniques. Notwithstanding these potential negatives, a number of in vivo tests are nearly always required as a prelude to a smaller number of animal performance trials. An in vivo evaluation may not however be possible in the prime target species, and a surrogate may be necessary as a first step, particularly if more than one target species has been identified. Palatability, mortality, feed conversion and growth must be evaluated. This type of evaluation will allow an economic value to be ascribed to the product in question, and therefore a cost benefit analysis of the upgrading technology step can be completed.

NEXT: FITTING INTO FEED FORMULAS

The animal performance evaluation will yield data that will allow nutritionists and feed formulators to fully include the upgraded product into commercial formulas. The choice at that point is between an inclusion strategy that targets cost savings or one that focuses on performance enhancement, or a mixture of both. Many nutritionists will set up a formula to compare the conventional and enzyme products such that there will be a direct comparison. Alternatively, both products could be used as substitutes for a higher value ingredient, such as low temperature (LT) fish meal, and the costs of diets compared. This approach has great value if the formulator is able to make equally nutritious diets that give equal performance. Here the full economic value change can be determined under realistic commercial conditions.
Sustainability is particularly important if the conventionally-manufactured product is prohibited (in extreme cases) or restricted to a lower level that does not allow use of all of the product. Notwithstanding economics, if enzyme upgrading allows full utilisation of a product, this can be seen to improve sustainability of the whole livestock sector.

The proof of any economic advantage can only come after performance trials have been completed and the improvements indicated by previous evaluations have been proven. Under these circumstances, the enzyme technology can be endorsed as providing the expected benefits. In the case of advantages both at the process and the inclusion (in feed) level, then the economic advantage may be shared, according to the local circumstances.

**Case study: Enzyme hydrolysed feather protein for aquaculture feeds**

**PROBLEM TO BE SOLVED**

Hydrolysed feather protein has in the past been poorly regarded by nutritionists around the world. Its status as a variable product with low digestibility and an absence of essential amino acids has probably justified this opinion in the past. However on the positive side, the high level of crude protein, and high levels of some key amino acids might make it a valuable ingredient for certain target species.

Feather protein is comprised of keratin, a complex polymer that is difficult to solubilise. As a result, typical processing using steam to hydrolyse the feather often results in an overprocessing which destroys part of the chemical constituents.

In consideration of the nature of feather protein it was decided to offer maximum upgrading potential. A stepwise approach was adopted, commencing with laboratory studies and continuing through pilot plant tests and into full-scale production. The trial goal of targeting aquaculture feeds as the most appropriate use of the upgraded protein was kept in mind from the early stages of the project.

**TARGET SUBSTRATE**

Protein is the major component of feathers comprising ~85% of the dry matter, but oil/waxes are also naturally present at ~5%. These fatty components may interfere with protein hydrolysis, so their presence must be dealt with by use of appropriate enzymes or surfactants. In the case of keratin protein, there are numerous sulphur bridges. A reducing compound may be effective in assisting the enzyme to break down these structural bonds.

**PROCESS PARAMETERS**

In the case of feather proteins there are a range of conventional or steam hydrolysis systems in use around the world. In most cases the most effective way forward has been to adapt conventional systems by addition of an extra step or by simply altering the processing profile.

**LABORATORY AND PILOT SCALE PRELIMINARIES**

During the development of the enzyme system key process parameters must be controlled. The following parameters were evaluated in laboratories such as the Alltech Bioscience Centers followed by pilot plant work, initially at PDM Group, Ltd. in the UK.

Feathers are naturally present in a wide variety of shapes and sizes, ranging from small down-type feathers from broilers to large primary turkey wing feathers up to 18 inches long. The development work has concluded that no pre-size reduction is necessary, as long as all other parameters are met satisfactorily.

Process temperature and time are vital parameters. Temperature must be controlled below a maximum of 55°C. A target of 50°C is recommended, for a time of 30 minutes. If a temperature of 50°C is not possible, then 40°C for 60 minutes or 30°C for 120 minutes are viable alternatives.

A dose of 0.5 kg of Allzyme™ FD (Alltech Inc.) plus 2.0 kg of Allzyme™ CoPack per tonne of raw feather is recommended. This addition rate was established following a series of dose response experiments.

The method of mixing should be able to distribute the enzyme and Allzyme™ CoPack throughout the feathers. A ribbon blender type design is particularly suitable, either as integral to the batch processor (as used in this study) or as a separate unit in a continuous system.

An adequate level of moisture is required to provide the appropriate water activity. In the case of feathers the natural moisture content is about 70% after removal from the bird and transporting around the slaughterhouse using a water fluming system. Some operators press excess water out of the feathers,
resulting in a moisture level of ~50%. However this is still an adequate level of moisture for enzyme action to take place.

Sterilization is important for hygiene standards to be attained, and a pressure / temperature combination of 2 bar/122°C for 20 minutes is sufficient to kill pathogenic microorganisms, such as *Salmonella* spp., and also vegetative states of bacteria such as *Clostridia* spp.

The moisture level following sterilisation is similar to the starting level, so dehydration is required to stabilise the product to ~5% moisture. There are several choices, including indirect steam drying or direct air drying. There is anecdotal evidence that the latter is preferable in terms of enhanced nutritional value, but the former may have advantages in terms of lower energy demands and the fact that environmental controls of lower volumes of air are somewhat easier.

For the enzyme process to be introduced into a full scale batch production, only changes to the processing profile will, in all probability, be necessary. As a result, no additional investment in plant and machinery is required. For continuous systems an additional enzyme process step is required which takes the form of an enzyme pre-hydrolyser unit. Both systems are outlined in Figure 1.

**EVALUATING RESPONSES**

In the case of enzyme processing of feathers there are two main desired effects. Firstly there are process and environment benefits, and secondly there are nutritional improvements.

Process and environmental responses include significantly lower energy inputs required, as can be seen by the contrast in time and temperature profiles in Figure 2. The lower input of energy in the enzyme process also results in lower environmental pollution because there is less breakdown of the nitrogen and sulphur-containing protein. As a consequence, lower levels of polluting chemicals such as ammonia and hydrogen sulphide need treating by the environmental control equipment, resulting in lower environmental control costs.

Nutritional evaluation requires assumptions to be made which may or may not always be valid. A major issue arises for feather protein when it is assumed that *in vitro* and *in vivo* measurements of nutritional value are positively related. This is not the case for feather protein digestibility and therefore only *in vivo* measurements are valid (Woodgate, 2004a). In addition, much preliminary work was necessary in a species that was not expected ultimately to be the major target species. For example, in the case of feather proteins, much *in vivo* evaluation was completed in chickens, although the substrate is not expected to feature in poultry diets in the future. The typical analytical profiles of hydrolysed feather proteins compared to fish meal, including *in vivo* data, are shown in Table 1.

In the case of hydrolysed feather protein, legislative limitations must be considered. In particular, the European Union (EU) regulation on the use of animal by-products (OJEC, 2002) is a limitation to utilization both in Europe and in countries that export to Europe. Woodgate (2004b) describes the practical implications of this regulation.
Creating alternative protein sources for aquafeeds using applied enzyme technologies

Table 1. Typical analysis of enzyme- and steam-hydrolysed feather proteins and fish meal1.

<table>
<thead>
<tr>
<th></th>
<th>Steam-hydrolysed</th>
<th>Enzyme-hydrolysed2</th>
<th>Fish meal</th>
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</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
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<td>8.5</td>
<td>6.5</td>
</tr>
<tr>
<td>True ME, MJ/kg</td>
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<td>15.5</td>
<td>12.5</td>
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<td>Phosphorus, %</td>
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<td>0.35</td>
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<tr>
<td>Digestible amino acids</td>
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<td>1.5*</td>
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<td>Arginine, %</td>
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<td>3.8*</td>
<td>3.6</td>
</tr>
</tbody>
</table>

1In vivo determination in poultry of feather protein sources (not fish).
2Allzyme™ FD.
*Derived from in vivo data.

Response to feather protein meal in aquaculture

Hydrolysed feather protein (HFP) in aquaculture feed meets both the requirements of the EU regulation and potentially offers a supply of highly digestible amino acids to a farmed species which has a particularly high demand for them. Preliminary digestibility studies were completed in rainbow trout (Serwata et al., 2004) and a comparison of enzyme-hydrolysed HFP with steam-hydrolysed HFP, together with a reference protein (low temperature fish meal, LTFM). Table 2 indicates the in vivo digestibility of protein and Table 3 shows the in vivo digestibility values for essential amino acids for all three protein sources.

The digestibility data obtained in this trial were then used to formulate a series of diets for rainbow trout that were isonutritive in terms of crude protein, digestible amino acids and energy. The growth trial was completed over 10 weeks, with the increase in live weight over the period shown in Figure 3. There were no significant differences in growth between

Figure 2. Comparison of standard and enzyme-hydrolysed feather protein processes.
the treatments, thus confirming the *in vivo* nutritional value determinations, and most importantly indicating that successful formulation based upon digestible amino acids is both practical and feasible. This allows more precision in formulation and lowers the waste protein in the diet that could be a potential source of pollution. Table 4 shows a comparison of the supply of indigestible protein (from digestibility data) which, when supplying equal amounts of digestible protein, reveals a significant difference between the steam-hydrolysed and enzyme-hydrolysed feather protein meal. These differences are important, as minimising the potential for nitrogen pollution is now critical for the aquaculture industry.

![Graph showing liveweight gain](image)

**Figure 3.** Effect of feather meal processing method on liveweight gain of rainbow trout.

Feeding trials on two tilapia species have been completed at the University of Plymouth in the UK (Davies and Fasakin, 2004), and preliminary data are shown in Tables 5 and 6. In both of these species, a positive response to the inclusion of enzyme-hydrolysed feather protein was observed when compared with the conventional steam-hydrolysed feather protein. Increased weight gain and improved FCR was seen with the diet including the enzyme-prepared product; and this improvement is currently being converted into economic value.

**Table 4.** Feed trial formulations including substitution of fish meal protein with feather protein and the indigestible protein content of the diet (kg/mT).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Fish meal</th>
<th>HFP</th>
<th>Digestible protein</th>
<th>Indigestible protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal + Enzyme HFP</td>
<td>600</td>
<td>200</td>
<td>363</td>
<td>33</td>
</tr>
<tr>
<td>Fish meal + Steam HFP</td>
<td>200</td>
<td>200</td>
<td>363</td>
<td>41</td>
</tr>
</tbody>
</table>

**Table 5.** Summary of feeding Trial 1: Tilapia (*O. niloticus*).

<table>
<thead>
<tr>
<th>Diet (inclusion)</th>
<th>Enzyme HFP</th>
<th>Steam HFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal (low temp), g/kg</td>
<td>146</td>
<td>146</td>
</tr>
<tr>
<td>Enzyme HFP, g/kg</td>
<td>243</td>
<td>243</td>
</tr>
<tr>
<td>Protein in diet, %</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Performance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start weight, g</td>
<td>5.46</td>
<td>5.56</td>
</tr>
<tr>
<td>Final weight, g</td>
<td>22.13</td>
<td>16.36</td>
</tr>
<tr>
<td>Weight gain, %</td>
<td>305</td>
<td>194</td>
</tr>
<tr>
<td>FCR</td>
<td>1.84</td>
<td>2.17</td>
</tr>
<tr>
<td>PER</td>
<td>1.49</td>
<td>1.11</td>
</tr>
</tbody>
</table>

**Table 6.** Summary of feeding Trial 2: Red tilapia (*O. niloticus x O. mossambicus*).

<table>
<thead>
<tr>
<th>Diet (inclusion)</th>
<th>Enzyme HFP</th>
<th>Steam HFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal (low temp), g/kg</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>Enzyme HFP, g/kg</td>
<td>224</td>
<td>224</td>
</tr>
<tr>
<td>Steam HFP, g/kg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Protein in diet, %</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Performance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start weight, g</td>
<td>2.03</td>
<td>1.91</td>
</tr>
<tr>
<td>Final weight, g</td>
<td>6.03</td>
<td>5.18</td>
</tr>
<tr>
<td>Weight gain, %</td>
<td>190</td>
<td>170</td>
</tr>
<tr>
<td>FCR</td>
<td>2.17</td>
<td>2.20</td>
</tr>
<tr>
<td>PER</td>
<td>1.30</td>
<td>1.33</td>
</tr>
</tbody>
</table>
Further research is ongoing in a range of other freshwater and marine species, including sea bass, sea bream and turbot. Plans are to extend studies to shrimp and eel in the future.

Conclusions

The advent of practical enzyme technology for the upgrading of key raw materials for farm animal feeding has given great opportunity for new or advanced products to be introduced into aquaculture feeds. The benefits of the enzyme technology as applied to the processing of feathers has given rise to a sustainable technology that gives economic benefits to both process and product alike. The introduction of formulation techniques that use digestible amino acid data has been beneficial for the enzyme processed product, which contains significantly higher levels of most digestible amino acids as measured \textit{in vivo}. This nutritional enhancement can be directly translated to economic benefit, which can be used in either the poultry or aquaculture sectors.

Using the techniques described above, many more animal or vegetable raw materials should be evaluated for potential upgrading with enzyme technology. These materials could include alfalfa, shrimp shells/ heads, fish heads, distillers dried grains, and sugar beet pulp. A programme to evaluate some of these feed materials is currently underway at Alltech Bioscience Centres.

References


Introduction

The world population is on the increase, as is the demand for aquatic food products. Production from capture fisheries at a global level is levelling off and most of the main fishing areas have reached their maximum potential. Global fish supply could be increased through reduction of discards and better use of by-catch for human consumption, e.g., use of at least part of the catch going for reduction to fish meal and fish oils. Better management of fishery resources and enhanced efforts to protect fishery resources from accelerating environmental degradation may well contribute to sustained, if not enhanced, fish supplies in the medium- to long-term.

Aquaculture appears to have stronger potential to meet the increasing demands for aquatic products in most regions of the world. Potential contributions from aquaculture to local food security and livelihoods can be highly significant, especially in many remote and resource-poor rural areas. The challenge is to develop approaches to increase the contribution of aquaculture, which are realistic and achievable, within the context of current social, economic, environmental and political circumstances. Such approaches should not focus only on increasing production; they should also focus on producing a product that is affordable, acceptable and accessible to all sectors of society.

Global aquaculture production and trends

Aquaculture is the fastest growing food-producing sector in the world. A great proportion (over 90%) of this production comes from the developing world. In contrast to terrestrial farming systems, where the bulk of global production is based on a limited number of animal and plant species, the aquaculture sector comprises of over 200 different species. This large number of species cultivated reflects the diversity of the sector, particularly the wide variety of candidate species cultivated and different production systems used. Currently all major aquaculture producing countries are in Asia. Six Asian countries (China, India, Indonesia,
Japan, Bangladesh and Thailand) contributed 83.3% to the global production in the year 2002.

The main species groups reared in fresh water are finfish while high value crustaceans and finfish predominate in brackish water, as molluscs and aquatic plants do in marine waters. Of these three environments, freshwater aquaculture could be considered as the most important in terms of contributing to achieving food security. Marine aquaculture, particularly of sea weeds and molluscs, also contributes to food security and poverty alleviation, as most of its products are produced within small to medium scale operations. Albeit brackish water shrimp culture is generally aimed at producing a high value export commodity, coastal shrimp culture also plays an important role in rural livelihoods and food security.

Fish, human health and nutrition

Fish is an important part of the diet for a large proportion of the people living in the developing world. Many types and forms of fish and aquatic products are available at affordable prices in developing countries. Economic affordability is a key factor as to why aquaculture is making an essential contribution to human health in the developing world. More ‘food fish’ is consumed globally on a per capita basis than any other type of meat or animal protein. In terms of animal protein supply, food fish represented 16.5% of total supply in 1997 (total global animal protein supply was reported as 27.1 g daily per capita in 1997), followed by pig meat (14.7%), beef and veal (13.6%), and poultry meat (12.5%). It is interesting to note that farmed aquatic meat production in China currently ranks second to pig meat; per capita availability of food fish in China increasing from 6.3 kg in 1984 to 25.5 kg in 1998.

The main reason for the high demand for staple food fish within most developing countries is their greater affordability to the poorer segments of the community. At present food fish represents the primary source of animal protein (contributing more than 25% of the total animal protein supply) for about 1 billion people within 58 countries worldwide, including many developing countries and LIFDCs (value excludes China).

In the diets of many countries, fish contributes more than or close to 50% of total animal proteins (e.g. Gambia, Ghana, Equatorial Guinea, Indonesia, Sierra Leone, Togo, Guinea, Bangladesh, the Republic of Congo, Cambodia, Sri Lanka, Philippines). The International Conference on Sustainable Contribution of Fisheries to Food Security, held in Kyoto in 1995, recognized that aquatic products contribute meaningfully to the maintenance of good nutrition.

Fish are important sources for many nutrients, including protein of very high quality, retinol (Vitamin A), vitamin D, vitamin E, iodine, selenium. Evidence is increasing that the consumption of fish enhances brain development and learning in children, protects vision and eye health, and offers protection from cardiovascular disease and some cancers. The fats and fatty acids in fish, particularly the long chain n-3 polyunsaturated fatty acids (n-3 PUFA), are highly beneficial and difficult to obtain from other food sources.

Increasing the worldwide availability of good quality animal products is necessary if the per capita supplies are to keep pace with the increase in demand. Aquaculture has an important role to play in this effort to fulfil the demand for animal products. Despite a lack of quantitative information concerning the role of rural aquaculture in achieving food security, there are undoubtedly benefits related to fish consumption; a) fish have a highly desirable nutrient profile, b) fish is an excellent source of high quality animal protein and with variable amounts of dietary energy, c) fatty fish, in particular, are an extremely rich source of omega-3 PUFAs, fat soluble vitamins (A, D and E) and water soluble vitamins (B complex), and d) fish is an important source of minerals (calcium, phosphorus, iron, iodine and selenium).

Fish, food security and rural development

Hunger and malnutrition remain amongst the most devastating problems facing the world’s poor. A considerable portion of the global population is currently suffering from one or more forms of nutrient deficiency. This remains a continuing travesty of the recognized fundamental human right to adequate food and nutrition, and freedom from hunger and malnutrition, particularly in a world that has both the resources and knowledge to end this catastrophe.

Latest estimates from FAO’s Report on the State of Food Insecurity 2002 indicate that 790 million people in the developing countries (840 million worldwide) regularly go to bed hungry. This is only about 20 million people less than the benchmark figure
set eight years ago by the World Food Summit and is far short of the pace needed to reduce the number of hungry people by half by 2015. Of the 11 million children under the age of five who die each year, more than half (6 million) die of malnutrition and hunger-related causes. The challenge is to rapidly accelerate the pace by which hunger and malnutrition are eliminated. This goal can be accomplished by improving access to food by the poorest and nutritionally most vulnerable population groups and individuals. This needs to be done in a manner that is socially acceptable and environmentally sustainable. This requires a dual approach whereby increased production and productivity gains are combined with improvements in the use and management of natural resources while also ensuring a more equitable access to food and other resources by the poor.

The objective of rural development is to facilitate a sustainable rural economy and to secure improvements in the welfare of rural populations. The opportunities for the integration of fish farming into rural development are characterized by diverse aquatic resources and a wide range of stakeholders with diverse livelihoods. Objectives may further range from food production, income generation, and wild stock enhancement to recreation (ornamental fish or sport). The scale may be intensive commercial or subsistence management within developed and less-developed economies.

The aquaculture sector provides worldwide employment to millions of people. Total employment in the aquaculture sector is highest in China where almost 4 million people are employed full-time in aquaculture production, and the annual growth rate for aquaculture employment rate is reported as 6%. In many countries the average market prices of fish are lower than those of other animal products such as chicken, pork and red meat. Especially in Asia the low prices of aquaculture commodities such as carps and tilapias make fish highly accessible to even the poorest segments of the population.

Studies have shown that practicing aquaculture is economically feasible under many different circumstances. Many types of low-cost, low-risk, simple, aquaculture technologies have emerged in recent years. Comparative studies between rice, rice-fish and fish farming systems in sub-Saharan Africa demonstrated that farmers investing in aquaculture increased their household incomes considerably with only minor investments. In Europe, USA, China and other Asian countries the increases in production and the number of people active in aquaculture over the last decade have shown that aquaculture production systems ranging from extensive to highly intensive can be economically feasible.

Aquaculture and foreign exchange

International trade in seafood is a multi-billion dollar sector, with global volumes expanding from around $7 billion in 1976, to $55.3 billion in 2000. Developing countries dominate seafood exports, contributing over 50% to the internationally traded seafood, with developed countries accounting for 80% or more of the imports, and Asia supplying 50% of all seafood exports. Asia produced 80% of the world's farmed shrimp in 2000. Trade in live food fish, especially reef species, an increasing volume of which is from farms, is concentrated in Asia and is virtually a one-way flow from Oceania, Southeast Asia and South Asia to mainly China. The foreign exchange earned through international trading of aquaculture products is a considerable contribution to many national economies.

Major issues and challenges

Aquaculture is an income generating activity. However, rapid sector growth has, in some instances, outstripped planning and regulatory activities. As a result, many areas have seen a regulatory rebound, with disproportionate requirements as resource use conflicts have occurred, resource scarcities have become more constraining and demand for product quality and safety has increased significantly. Increasingly, some markets will consider additional product attributes, like environmental and social impacts of production. In some regions, aquaculture faces a considerable problem with public perception. In some cases, aquaculture development has failed to keep up with, or meet, many environmental and socio-economic issues and expectations. Future aquaculture development needs to produce a product which is not only acceptable to public and consumers in terms of price, quality, and safety, but also in terms of environmental cost.

Although it has been said that aquaculture has a significant potential for improving food security and alleviating poverty, the role of aquaculture in food security has been a major concern of the sector for many years. From the point of view of production, it has been in the increase for many years, although
From farm to fork: the challenges that fish farming faces

at a reducing rate. Overall, the driving force behind the relative increase in production and decline in value appears to be declining prices for luxury and commodity products as markets are becoming saturated and competition is increasing.

Maintaining environmental sustainability

Certain forms of aquaculture have a bad reputation. The argument mainly comes from the use of feed and seed resources, disease control and chemical and veterinary drug use, accumulation of environmental contaminants, escapes and point source contamination of wild resources, negative or low net energy conversion during farming of top carnivores, mangrove clearance and land degradation, etc. Some of the arguments are true and worthy of considering but the quantum to which the issues are highlighted is certainly biased.

Traditional or extensive aquaculture, which is a low-tech, low-input aquaculture practice invented by the Chinese some 3000 years ago, is still in practice, producing large volumes of fish feeding low in the food chain. Over 80% of the fish produced by aquaculture are herbivorous or omnivorous, mostly produced in low-intensity systems for local consumption. They, undoubtedly support livelihoods of people, provide food, alleviate rural poverty and improve health among less fortunate communities. However modern-day aquaculture, mainly the production of high value carnivorous fish or shellfish destined to import markets is a different kettle of fish altogether. This sector uses a considerable quantity of natural resources and also produces a considerable quantity of effluent and waste. The sector’s sustainability and environmental acceptability has been increased significantly over the past decade through research involving development of technically specialised conditions.

However, the environmental, social and economic landscape within which aquaculture has performed well up to now is changing. In particular, competition will increase as barriers to trade decline through the process of economic globalization. In addition, the negative environmental and social impacts of aquaculture that occur in some situations will increase public scrutiny and criticism that could well alter the policies that have so far fostered growth. The trend has been to improve the environmental acceptability or sustainability of the sector through several interventions and developments such as; a) reduced reliance on fishmeal in fish feed, b) increased efficiency in feed formulation, c) improving food conversion ratio thus increasing net energy conversion, d) containment and recycling of wastes in cages and flow-through systems, e) increased land and water use efficiency, f) improvement to health management and reduction of chemical and veterinary drug use, and g) domestication and genetic improvement towards reducing negative impacts on aquatic biodiversity.

While production is increased, the global population is also on the increase and the land and water use for aquaculture production is also increasing. Aquaculture production is increasing, not necessarily due to intensification but mainly due to expansion. The global increase in production, at an average rate of 9%, is mainly due to the increase in production rate in China; and if the trend is considered without China the rate is much less. Use of resources such as fish and fish products (fish meal) for producing fish is being questioned. The net energy conversion in certain forms of aquaculture appears to have negative ecological impacts thus requiring urgent rectification. Reduction in fish meal in fish feed requires more attention and research.

Keeping up with safety and quality

As mentioned earlier, international trade in seafood is a multi-billion dollar sector. Developing countries dominate seafood exports, contributing over 50% to the internationally traded seafood, with developed countries accounting for 80% or more of the imports. Asian developing countries top seafood production statistics. As the current production through capture fisheries is somewhat static and in order to respond to the increasing demand for seafood, aquaculture is now contributing significantly to global seafood trade and the share is increasing. While the quantity of trade is increasing, the consumer demand for safer seafood is also increasing and as a result the international trading environment is changing, with food safety issues in particular receiving considerable attention.

Traditionally, food safety in seafood has been concerned with post-harvest handling and processing. Now, with importing countries and consumers concerned about residues, attention has shifted toward the way seafood is produced. This requires aquaculturists to work together with food safety experts to develop systems for farming aquatic animals that assures food safety, (particularly the
species which are destined to international trade), based on internationally accepted, science-based quality control mechanisms, such as risk assessment and HACCP and Good Hygienic Practice (GHP). Food safety concerns are leading to new demands for traceability of aquaculture products. This will not be easy with the large number of small-scale farmers engaged in production, and the fragmented supply chains operating in many countries. Substantial institutional reorganization, legal and policy development, awareness raising and capacity building efforts will be essential among the diverse stakeholders in public and business sectors to make this work.

International food safety standards are being set with minimum inputs from the region, in particularly from the producing sector, for various reasons. Asia needs to enhance and organize better its inputs to international food safety standard setting bodies such as Codex Alimentarius, given the importance of such standards for future trade in aquaculture products from the region. With most countries in Asia giving increased attention to food safety, there is a growing proliferation of product certification systems, “good aquaculture practice” guidelines, Codes of Conduct, and other mechanisms/schemes intended to provide a basis for safe and sustainable seafood production. Without some harmonization among regional countries, this proliferation of certification schemes has potential to confuse consumers, importing countries, lead to increased costs, and potentially constrain trade.

Asian domestic and intra-regional trade in aquaculture products, services and inputs such as feed and chemicals, is growing, in line with increasing free-trade agreements between countries. This opens new opportunities for trade and development, perhaps helping to avoid some of the complex procedures of other importing regions, but also poses challenges. This further emphasizes the need for harmonization of food safety assurance procedures among trading partners in Asia. Such cooperation may also avoid problems of residues being transferred from one country to another.

Applying new food safety standards and traceability poses special organizational difficulties for the large community of small-scale farmers in the region, and as a consequence, some of the poorest farmers might be at risk due to difficulties in participating in such schemes. There is a need therefore to better understand the implications of new food safety standards and international trading standards for small-scale farmers, and develop suitable market-oriented solutions to the problems faced by the small-scale sector, allowing the sector to benefit from the development opportunities offered through trade, while reducing exposure to the associated risks.

Asian aquaculture systems have many traditional and diverse advantages in safe, healthy and sustainable seafood production, such as some ecologically sound integrated farming systems. Collaborative research and development should be used to encourage both the traditions and innovations in aquaculture farming that can give the region comparative advantage in this new trading environment.

**Combating disease and managing health**

Disease has become a primary constraint to sustainable aquaculture production and product trade. A multitude of factors has contributed to the health problems currently faced by aquaculture. As note above, over the years aquaculture has expanded, intensified, and diversified, based heavily on movements of live aquatic animals and animal products (broodstock, seed, and feed). This trend has been triggered by changing circumstances and perspectives, especially world trade liberalisation.

New outlooks and directions have accelerated the accidental spread and incursion of diseases into new populations and geographic regions, for example, through movements of hatchery produced stocks, new species for culture, enhancement and development of the ornamental fish trade. The impacts of trans-boundary aquatic animal diseases on international trade, as well as socioeconomic and biodiversity implications are considerable. Different measures are being used to deal with diseases of fish and shellfish, such as; international codes, regionally oriented guidelines, national programs and legislation, technology for diagnostics, therapy and information communication.

The aquatic animal health management programs carried out in different parts of the globe are different with respect to efficacy of disease prophylaxis/control and pathogen detection/disease diagnostics, inherent problems with national legislation and international/regional codes, and the effectiveness of programs on education, training and extension services. Health management problems which pose risks to rural small-scale aquaculture require special consideration. There is a need for effective communication at all levels of the production systems. There are roles to play by the state, private sector (e.g. aquaculturists,
industry associations, cooperatives, etc.) professional societies, diagnosticians and researchers, education, training, and other related extension services.

The current trend to meet the demand for more aquatic food, through expansion, intensification, and diversification, will continue to provoke the emergence and recurrence of disease challenges. How industry, government and other stakeholders rise to meet these challenges will dictate how aquaculture survives and achieves true sustainability. The options are not always easy. The varying levels of political, economic and social development among countries, the trans-boundary nature and commonality of many major disease problems, and the need to harmonise approaches, all complicate effective cooperation and consultation. The current situation offers a big challenge and an opportunity to all concerned but, if maintained at the present level, major epidemics will continue to threaten, break out and impact the ultimate goal of aquaculture sustainability.

Conclusions

Globalization, stringent food safety standards, realization of environmental and social responsibility of aquaculture production has begun to change the way aquaculture development and management has been pursued, in particular the practices which brought global controversy. The ‘aquaculture industry’ will benefit from this and noticeable changes will take place in countries where high value commodities are farmed, especially for foreign markets.

States will respond to improving legislation and regulatory frameworks for better practices, farmers themselves will get together to police and regulate their practices, in the face of less state regulation. Markets and profits will continue to drive the international trade and as in agriculture sector, larger and more vertically integrated systems will increase their share of the international market. Maybe small numbers of large producers will eventually produce huge quantities of a few species destined for foreign markets. Smaller farms, to make profits, will have to look for specialised products. However, as global economy increases, the viability of these small-scale, profit-oriented farms will increase and will benefit from parallel development of the corporate sector, thus ensuring market synergy and co-existence.

Aquaculture will continue to grow but must address the costs of production, quality and safety of products, international trade obligations and requirements, environmental concerns, etc. More emphasis on investment, research, information, and public education are needed. Challenges for increasing aquaculture’s contribution to food security, poverty alleviation and rural livelihoods will have to be met. Aquaculture development, especially if it is to be sustainable for food security goals, may need to be stimulated, at least in the beginning, so there should be a key point on increasing access to credit for farmers, producers and local marketing. It is important to understand the investment opportunities in the sector.

In an era of globalisation, it is imperative to emphasise national and international trends of trade. Trade of aquaculture produce, input supplies, capital and information are all important to acknowledge. Aquaculture is dependent on key natural resources such as water, land, seed, and nutrients. There is strong pressure for production and marketing systems that are more efficient and more effective in terms of resource utilisation. In this respect, we should invest in research on developing production and marketing systems with better resource utilisation and more efficient performance.

During production, there should be emphasis on targeting the consumers. We must emphasise the difference between mass production and production for the masses. For example, trends of formerly expensive produce such as salmon and shrimp are increasingly becoming affordable by larger segments of the population. We should compete with and complement other food producing sectors and providers. Aquaculture produce should be acceptable to all sectors of the society. Tremendous gains will be possible through improved biotechnology, genetic modification, improved nutrition, probiotics, disease diagnosis and treatment. However, the problem of consumer resistance to perceived risks stemming from ‘unnatural’ products, ethical problems and fear of unknown technologies will affect potential gain.

As mentioned, environmental and human health issues will slow development or reduce market access. Strategic solutions are required. We should emphasise biosafety issues, development and promotion of biotechnology that conserve the environment; we should promote policies that support ethical issues of welfare and autonomy and emphasise labelling and transparency for production process and beneficiaries. There is a need to increase the impact of research to understand technical and other
constraints and to enhance the applicability and use of research results in the development of strategies to overcome these challenges.

References


Companion animals
USA poultry meal: quality issues and concerns in pet foods

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Introduction

Poultry protein meal represents a substantial portion of the high quality protein and fat in modern companion animal diets. Poultry protein meal is commonly included at 5 to 40% and can contribute in excess of 85% of the dietary protein and 30% of the dietary fat. Thus, changes to the quality or composition of the protein or fat in poultry protein meal can have profound effects on the nutritional value of the diet. The process of producing poultry protein meal is a daunting task undertaken to stabilize the raw material from microbial deterioration. Unfortunately, during this process protein and fat quality are affected.

Defining poultry meal

The availability of fresh poultry and rendered poultry products coincided with the commercialization and industrialization of poultry production in the 1940s and 1950s; and feed values for poultry by-product meal were first established in the 1950s (Fuller, 1996). The volume of rendered poultry proteins in 2003 was estimated at 3,073.5 million lbs; and on average the companion animal industry consumes about 23% (Pearl, 2003).

The collective term ‘poultry protein meal’ covers both poultry by-product meal and poultry meal from chicken, turkey, or other poultry origin. The official definitions according to the Association of American Feed Control Officials (AAFCO, 2004) are:

9.10 Poultry By-Product Meal consists of the ground, rendered, clean parts of the carcass of slaughtered poultry, such as necks, feet, undeveloped eggs, and intestines, exclusive of feathers, except in such amounts as might occur unavoidably in good processing practices. The label shall include guarantees for minimum crude protein, minimum crude fat, maximum crude fiber, minimum phosphorous (P), and minimum and maximum calcium (Ca). The calcium (Ca) level shall not exceed the actual level of phosphorous (P) by more than 2.2 times. If the product bears a name descriptive of its kind, the name must correspond thereto.

9.71 Poultry Meal is the dry rendered product from a combination of clean flesh and skin with or without accompanying bone, derived from the parts of whole carcasses of poultry or a combination thereof, exclusive of feathers, heads, feet, and entrails. It shall be suitable for use in animal food. If it bears a name descriptive of its kind, it must correspond thereto.

In short, poultry by-product meal (PBPM) differs from poultry meal (PM) only by the inclusion of heads, feet, and entrails. However, no quality considerations are described in the definitions. A proposal in 1998 to drop the ‘by-product’ designation from poultry products and replace it with the term ‘protein’ was rejected by AAFCO. The newest proposal requests that the ‘by-’ be dropped from by-product. Proponents of this request are asking for a level playing field with meat meal, fish meal, and lamb meal; while opponents claim that this would be misleading to the consumer. At issue is the name on a label and how it is represented. However, there is little evidence in the literature to support differing names. In a comparison of chicken meal (CM) and chicken by-product meal (CBPM) using a chick
protein efficiency ratio (PER) model, no difference was observed in protein quality (Aldrich and Daristotle, 1998). Bednar et al. (2000) fed dogs diets containing either PBPM or PM and reported that ileal digestibility of protein and amino acids was not different between treatments; however, total tract protein digestibility was 6.74% lower for dogs receiving PBPM than for dogs receiving PM. From the perspective of chemical composition, there is a fair amount of overlap between PM and PBPM (Table 1).

Several different grades of rendered poultry products are available. Feed grade is seldom used in pet food because it contains a higher level of ash and lower protein content. Standard pet food grade contains less than 14% ash; and low ash poultry meal and (or) poultry by-product meal contain less than 11% ash. The latter is available in limited quantities at a premium price and typically reserved for low ash cat formulas (Miller, 1996).

## Rendering

‘Rendering’ is required to stabilize the mass of poultry co-products that have been removed from the human edible stream. The rendering plant accepts and processes all raw materials received from the animal processing plant and must ‘render’ them stable so as to avoid public health problems. The process transforms raw unused poultry parts into a form that can be easily stored and transported. Rendering, in its simplest description, is a sterilization, dehydration, and resizing process (Miller, 1996). In the US, standard rendering is a ‘high temperature’ process. This involves extensive heating (approximately 280°F), which drives water and fat from the bone and tissue. The fat is removed by pressing and the remaining ‘cake’ is ground in a hammer mill to a uniform particle size. The fat goes to storage vessels where it can be further processed or sold. Likewise, the ground meal is conveyed to storage silos for cooling and eventual sale, or further processed in an effort to ‘improve’ its chemical composition.

## Protein quality

Fresh meats would be a preferred material with which to construct petfood diets, but this is not always practical for several reasons: 1) expense associated with freezing and chilling, 2) expense involved with

| Table 1. Nutrient composition (dry matter basis) of poultry meal (PM) and (or) poultry by-product meal (PBPM) and low ash (LA) PBPM or PM. |
|-----------------------------------|------------|------------|------------|------------|------------|
|                                   | PBPM       | PBPM       | PBPM-LA    | PM-LA      | PM         |
|                                   | Murray     | Johnson    | Johnson    | Clapper    | Yamka      |
| Protein                           | 67.6       | 64.6       | 68.8       | 74.5       | 69.3       |
| Ash                               | 13.9       | 17.0       | 7.5        | 9.6        | 9.0        |
| Fat                               | 11.6       | 12.5       | 19.8       | 15.0       | 11.6       |
| Essential amino acids             |            |            |            |            |            |
| Arg                               | 4.4        | 4.6        | 4.8        | 4.9        | 4.4        |
| His                               | 1.6        | 1.2        | 1.5        | 1.8        | 1.7        |
| Ile                               | 2.6        | 2.3        | 2.9        | 2.8        | 2.3        |
| Leu                               | 4.6        | 4.4        | 5.4        | 5.2        | 4.8        |
| Lys                               | 3.8        | 3.6        | 4.2        | 4.7        | 3.1        |
| Met                               | 1.1        | 1.2        | 1.4        | 0.8        | 1.1        |
| Phe                               | 2.5        | 2.6        | 3.0        | 2.8        | 2.7        |
| Thr                               | 2.5        | 2.6        | 3.1        | 2.8        | 3.0        |
| Trp                               | NR         | NR         | NR         | NR         | 0.5        |
| Val                               | 3.1        | 2.9        | 3.6        | 3.3        | 3.2        |
| Nonessential amino acids          |            |            |            |            |            |
| Ala                               | 4.1        | 4.5        | 4.5        | 4.4        | 4.3        |
| Asp                               | 5.2        | 5.8        | 6.3        | 6.3        | 5.6        |
| Cys                               | 1.8        | 0.9        | 1.0        | 1.0        | 1.3        |
| Glu                               | 8.5        | 8.7        | 9.8        | 9.7        | 8.6        |
| Gly                               | 5.7        | 7.3        | 5.6        | 5.5        | 6.5        |
| Pro                               | 5.3        | 5.0        | 4.4        | 4.4        | NR         |
| Ser                               | 2.7        | 3.1        | 3.4        | 3.2        | 4.4        |
| Tyr                               | 1.8        | 1.7        | 2.3        | 2.1        | 1.8        |

NR: values not reported
transportation of high amounts of moisture, 3) most extrusion processes will not handle more than 25% fresh meat in a formula, 4) fresh meat reduces production efficiency, and 5) fresh meat diets can be more difficult to stabilize. Therefore, the use of dry meals with concentrated protein is often necessary.

Achieving this dry meal requires rendering; however, the rendering process can have a substantial impact on nutritional quality. Murray et al. (1997) reported that protein and total amino acid digestibility at the ileum in dogs fed a diet containing rendered poultry by-product (meal) was reduced by greater than 10% when compared to a diet containing fresh poultry by-product. However, no differences in total tract protein digestibility were detected. Energy and amino acid digestibility of animal by-product meals can be negatively affected by different rendering processes, high rendering temperatures, extended residence times (Wang, 1997), and high rendering vessel pressures (Shirley and Parsons, 2000).

Poultry protein meals can have a better (Bednar et al., 2000; Yamka, 2003b), equal (Bednar et al., 2000), or poorer (Clapper et al., 2001) protein digestibility than soybean meal. Unlike vegetable proteins, poultry meal is not fraught with some of the anti-nutritional components; however, composition (Locatelli and Hoehler, 2003; Dozier et al., 2003) and performance can be quite variable. As an example, van Kempen et al. (2004) reported that the variability in digestible lysine and methionine in PBPM was 3 times that of soybean meal. Locatelli and Hoehler (2003) reported that protein and amino acid concentrations of 409 PBPM samples from 1999 to 2002 varied widely within years and the mean concentrations changed by several percentage points from one year to the next.

From where does this variability arise? Besides variation in processing conditions, the components making up the raw material mix can also change from day to day, week to week, and season to season. To illustrate this point, one must look at the protein quality of the different raw material components. In a study comparing protein quality of various rendered chicken parts, Aldrich and Daristotle (1998) reported that the PER value of feet and bone/cartilage were 0.87 and 1.22, respectively; whereas heads, viscera, and gizzards/livers/hearts were 2.50, 3.04, and 3.08, respectively. The protein quality of these latter parts was comparable to backs/breastplate and whole birds without feathers (2.88 and 3.43, respectively). Based on these data, one might conclude that the level of ash (bone residue) would have the greatest impact on nutritional quality of the meal. However, ash level in PBPM (16.3% vs. 7.2%) did not affect ileal digestibility of protein or amino acids or total tract protein digestibility in dogs, amino acid digestibility in cecectomized roosters (Johnson et al., 1998), or protein quality (PER) in chicks (Johnson and Parsons, 1997). Feeding dogs increasing amounts of low ash PM (10.4% to 32.5% of the diet) did not affect protein or amino acid digestibility at the ileum (Yamka et al., 2003a). Thus, increasing consumption of ash from poultry sources does not negatively affect nutrient quality. If not ash, then it is likely that the lower protein quality of bone/cartilage and feet is associated with high levels of connective tissue and a reduction in the ratio of essential to non-essential amino acids. Bone/cartilage can be a component of either PM or PBPM, and feet a component of PBPM; regardless, adding these components to the raw material mix would likely reduce the quality of either meal.

To assure the protein quality of poultry protein meal, the focus of the pet food manufacturer should be on a solid relationship with the supplier/renderer and an understanding of their business processes. Measurement of amino acids and amino acid digestibility and utilization can be a good source of information for general trends in quality; however, this is too slow, laborious, and costly for day-to-day decisions. Numerous tests have been described to provide rapid estimates. They range from nitrogen solubility tests in various buffers, to in vitro tests with enzymes and acids, to the use of Near Infrared Reflectance (NIR) and Fourier Transform Infrared Reflectance (FTIR) technology (van Kempen et al., 2004). Each provides information supporting purchase and use of consistent poultry protein meal; however, the rapid methods must be validated periodically with animal test data.

**Fat quality**

Fat in dog and cat diets is used to support the energy needs of the animal, meet its essential fatty acid requirements, aid absorption of fat soluble vitamins, impart flavor, aroma, and texture to the product, and enhance product appearance. Poultry meal contains about 15% fat, the portion that is left after the extraction process. In one of the few reports in the literature for PBPM, the predominant fatty acids were oleic (18:1n-9), palmitic (16:0), and linoleic (18:2n-6) at 41, 21.7 and 20%, respectively (Table 2; Kirkland and Fuller, 1971). This agrees fairly well with the poultry fat data of Pesti et al. (2002) and
the USDA National Nutrient Database (USDA-ARS, 2003). Whether there is a real difference between the fatty acid profiles of poultry protein meal and poultry fat has not been reported; but one might speculate that the structural lipids remaining in poultry protein meal would be slightly different than those present in bulk poultry fat. Differences between chicken and turkey fat appear to be small. Chicken fat is comprised of 65.6% mono- and poly-unsaturated fatty acids with 37.3% as oleic and 19.5% as linoleic compared to turkey fat with 66% as mono- and poly-unsaturated fatty acids and 35.9% as oleic and 21.2% as linoleic (USDA-ARS, 2003). It has been suggested that the proportion of linoleic acid has been increasing as more unsaturated vegetable fats are incorporated into poultry diets. This has implications for the nutritive value and the stability of the fat; however, no data were found in the literature to support this claim.

Table 2. Fatty acid profile of poultry by-product meal (PBPM), poultry grease, chicken fat, and turkey fat.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic (14:0)</td>
<td>1.3</td>
<td>0.61</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Palmitic (16:0)</td>
<td>21.7</td>
<td>22.83</td>
<td>21.6</td>
<td>20.6</td>
</tr>
<tr>
<td>Palmitoleic (16:1)</td>
<td>6.4</td>
<td>8.98</td>
<td>5.7</td>
<td>6</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>7.6</td>
<td>4.67</td>
<td>6</td>
<td>6.2</td>
</tr>
<tr>
<td>Oleic (18:1)</td>
<td>4.1</td>
<td>43.4</td>
<td>37.3</td>
<td>35.9</td>
</tr>
<tr>
<td>Linoleic (18:2)</td>
<td>20</td>
<td>16.88</td>
<td>19.5</td>
<td>21.2</td>
</tr>
<tr>
<td>Linolenic (18:3)</td>
<td>1</td>
<td>1.14</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>Arachidonic (20:4)</td>
<td>1</td>
<td>0.36</td>
<td>0.1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

The high level of linoleic acid present in poultry protein meal fat complements the nutrient requirements for dogs and cats (1% and 0.5% of diet DM, respectively). In addition, poultry fat is well accepted by both dogs and cats; and its flavor is preferred over a number of other fat sources. However, the fat present in poultry protein meal is susceptible to oxidative rancidity. Rancidity is the irreversible oxidation of fat initiated by catalysts such as light, transition metals (iron and copper), heat, and free radicals (molecules possessing an unpaired electron; Figure 1). Once the reaction is initiated, it becomes autocatalytic and proceeds unabated until the reactants are completely exhausted. The point in time at which the rate of the reaction becomes autocatalytic corresponds to the induction point (IP) and from this point forward, the rate of the reaction proceeds rapidly (Figure 2). This rapid deterioration is classically termed propagation and concludes, as described, once the reactants are exhausted.

While heat, pressure, mechanical grinding, and mixing are necessary components of the rendering process, unfortunately they contribute to the course of fat oxidation. During rendering, the inherent animal cellular defense mechanisms are disrupted or destroyed. Lipids are intimately mixed with metabolic enzymes, transition metals, and water. Transition metals, such as iron, are key catalysts to initiate the oxidation process. Interestingly, chicken and turkey lose a greater proportion of heme iron during cooking than do the red meats (Lombardi-Boccia et al., 2002). These factors all combine to accelerate oxidation of the fat in the poultry protein meal.

Figure 1. Schematic of the catalyst induced (T° = temperature, uV = ultraviolet light, Fe = transition metals like iron and copper, and *R, \( \cdot O_2 \) = free radicals) oxidation of an unsaturated fat (R=R) to intermediate (\( \cdot R-R \)) and secondary (R’ and R”) oxidation products.
Monitoring fat quality depends upon gathering and utilizing data from several different methods. The elapsed time from raw material to finished product can have an impact on the production of free fatty acids (FFA). Free fatty acids are produced as a result of lipase enzyme activity on the triglycerides. The cleavage of fatty acid(s) from the glycerol backbone produces non-esterified or ‘free’ fatty acids. These FFAs are more susceptible to oxidation. The heat process of rendering denatures the lipase enzymes, thus monitoring FFA levels provides an indication of pre-rendering raw material handling. The initial peroxide value (iPV) is commonly measured. However, its relevance is valid only as an initial post-rendering indication of quality. This is because peroxides break down to secondary oxidation products. The secondary oxidation products include aldehydes, ketones, epoxides, etc. It is these secondary oxidation products that result in rancidity and off-odors. Standard methods for their quantification include p-anisidine values, GC-headspace analysis, and reaction with thiobarbituric acid (TBARS). It is these secondary oxidation products and other reactive oxidation species that can be detrimental. As an example, consumption of oxidized poultry fat has been reported to decrease growth, lower hematocrit counts, decrease half-life of intestinal cells, and affect IgA and lymphocyte proliferation in chicks and pigs (Dibner et al., 1996). Feeding diets with moderate levels of aldehydes was also shown to retard puppy growth and suppress immune function (Turek et al., 2003). In many cases, rancidity in the diet can be overcome with high levels of supplemental vitamin E. However, this is an expensive solution and one that neglects the root problem.

The nutritive value of poultry fat is compromised with elevated levels of oxidation; wherein, the essential fatty acids and energy value have been shown to decline over time. The proportion of linoleic acid in unstabilized PBPM fat declined from 20% to 11.8% over a 12-week storage period and corresponded to an elevation in peroxide values (PV; Figure 4; Kirkland and Fuller, 1971). In this study, linoleic acid levels did not change for stabilized PBPM. Likewise, linoleic acid declined in puppy diets and subsequently in serum as the level of dietary aldehydes increased (Figure 3; Turek et al., 2003). In poultry grease, an elevated level of oxidation was associated with lower dietary metabolizable energy (Pesti et al., 2002). There have been suggestions that the fatty acid concentrations and oxidative conditions of poultry protein meal vary with season. This may be possible as seasonal temperatures, poultry feeding practices, and holiday poultry consumption changes; however, no reports were found in the literature that supports this claim.

Oxidation of fat can be retarded, but not eliminated. The goal of most poultry protein meal suppliers is to delay the onset of oxidation through good management practices, quick product turnover, and the judicious application of antioxidants early in the rendering process. The idea is to slow or delay the
IP for as long as possible. Poultry protein meal suppliers and some pet food manufacturers use the antioxidant ethoxyquin for this purpose. Ethoxyquin has been shown to be effective at reducing oxidation (Kirkland and Fuller, 1971), and is approved for use on animal feeds (21CFR573.380). Other synthetic antioxidants such as BHA, BHT, TBHQ, and propyl gallate are used separately or in various combinations with general success. However, synthetic antioxidants have fallen out of favor with purchasers of pet foods.

The newer challenge to stabilizing poultry protein meal is the request for natural preservatives. Over the past decade there has been a substantial increase in the number of products that carry a ‘natural’ claim. This eliminates use of the synthetic antioxidants mentioned previously. Poultry protein meal can be effectively stabilized with natural antioxidant.
systems; however, it is much more costly and requires more attention to the details. Most natural antioxidant systems are built on a backbone of mixed tocopherols carried in a vegetable oil with added chelators, spices, and emulsifiers. Because the natural antioxidants are oil miscible and mix poorly with water, there are other physical properties (e.g. viscosity) that make them more difficult to work with. The goal is to match the amount of natural antioxidant needed with the desired shelf-life of the poultry protein meal. Uniform application as early in the rendering process as possible and thorough mixing so that the antioxidant is intimately associated with the lipid in the meal is preferable. Constant monitoring of application systems and efficacy is required.

Rapid and (or) predictive analytical tests are available to assist in decision making about the relative freshness of poultry protein meal samples and the efficacy of the antioxidant system. Most of the methods rely upon an external factor to accelerate the rate of oxidation, i.e. heat, oxygen, or pro-oxidants, etc. The most appropriate tests for poultry protein meal are the Schaal oven, oxygen uptake (Oxygen Bomb), and pro-oxidant stress tests. Each of these methods requires the determination of the relationship to real-time storage. Once this relationship is known, they can provide very rapid and reliable information about the stability of poultry protein meal.

**Summary**

Very few studies have been reported in the literature relating changes in protein and fat quality of poultry protein meal to performance and health of dogs and cats. Because the rendering process by which the meal is produced requires heat and mechanical mixing, the protein and fat quality can be dramatically affected. The protein quality of poultry protein meal can be affected by heat damage and dilution with non-essential amino acids; and the fat quality can be negatively affected by oxidative rancidity. Each result in a lower than expected nutritional value associated with the poultry protein meal, and can have harmful effects on the dog and cat. It is recommended that constant monitoring to assure the quality of the protein and fat be practiced in coordination with the renderer/supplier. Further, judicious use of antioxidants and close monitoring of results will assure a safe and nutritionally adequate poultry protein meal for the companion animal industry.

**References**


The role of yeasts in companion animal nutrition

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Types of yeast

Several strains of the yeast *Saccharomyces cerevisiae* are used in the baking, brewing, distilling, and wine production industries (Sumner and Avery, 2002). Although these strains share common features such as efficient sugar utilization, high ethanol tolerance and production, high yield and fermentation rate, and genetic stability, they also possess properties specific to each group (Trivedi et al., 1986; Benítez et al., 1996).

Eight official and one ‘tentative’ yeast products are currently defined by the Association of American Feed Control Officials (2003) and are differentiated by source of yeast and characteristics such as moisture and crude protein concentrations, and fermentative activity. Brewer's dried yeast is the dried, non-fermentative, non-extracted yeast of the botanical classification *Saccharomyces* resulting as a by-product from the brewing of beer and ale. It must contain not less than 35% crude protein and be labeled according to its crude protein content (AAFCO, 2003). As defined, brewer's dried yeast must originate from a brewery and the brewing of beverages, beer or ale for human consumption, and should not be confused with corn wet milling yeast that is used in industrial ethanol production.

Brewer's dried yeast and corn wet milling yeast are different in terms of chemical composition and organoleptic properties, which is likely due to differences in the fermentation processes and in the substrates used (Table 1). In the brewing industry, wort derived mostly from malted barley is fermented slowly at temperatures of 10 to 20°C using a batch fermentation process that yields a beer with an alcohol content of approximately 6%. In contrast, during wet milling ethanol fermentation, distillers commonly grind and cook corn using enzymes to convert starch to sugar. A rapid continuous fermentation process at temperatures between 35 and 38°C then is employed to maximize substrate utilization and ethanol production yielding 9-12% alcohol. Although not fully researched, many of the differences in brewer's dried yeast and yeast from corn wet milling ethanol production [e.g., fat content, hops products (caryophyllene, humulene), and sugar profiles] are likely factors that affect palatability.

<table>
<thead>
<tr>
<th>Item</th>
<th>Brewer's yeast</th>
<th>Corn wet milling yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter, %</td>
<td>95.3</td>
<td>91.2</td>
</tr>
<tr>
<td>Organic matter, % of DM</td>
<td>94.2</td>
<td>92.8</td>
</tr>
<tr>
<td>Crude protein, % of DM</td>
<td>43.1</td>
<td>46.8</td>
</tr>
<tr>
<td>Total dietary fiber, % of DM</td>
<td>22.5</td>
<td>19.1</td>
</tr>
<tr>
<td>Ash, % of DM</td>
<td>5.9</td>
<td>7.2</td>
</tr>
<tr>
<td>Fat, % of DM</td>
<td>3.0</td>
<td>9.4</td>
</tr>
<tr>
<td>Total monosaccharides, mg/g (DMB)</td>
<td>460.8</td>
<td>277.8</td>
</tr>
<tr>
<td>Glucose, mg/g (DMB)</td>
<td>369.7</td>
<td>168.1</td>
</tr>
<tr>
<td>Mannose, mg/g (DMB)</td>
<td>81.4</td>
<td>60.6</td>
</tr>
<tr>
<td>Sugar alcohols, mg/g (DMB)</td>
<td>9.7</td>
<td>49.1</td>
</tr>
<tr>
<td>Uronic acids, mg/g (DMB)</td>
<td>7.0</td>
<td>7.2</td>
</tr>
<tr>
<td>Caryophyllene, µg/g</td>
<td>0.31</td>
<td>ND^1</td>
</tr>
<tr>
<td>Humulene, µg/g</td>
<td>0.65</td>
<td>ND</td>
</tr>
</tbody>
</table>

^1Not detected

COMPOSITION OF BREWER’S YEAST

Commercially available brewer's yeast is typically dried from a yeast slurry to a dry powder of less than 10% moisture to facilitate handling, storage, and transport. Brewer's yeast is relatively high in crude protein and carbohydrate concentrations, while the
concentrations of fat and ash are relatively low. This is not surprising because yeast synthesizes protein and vitamins while absorbing minerals from the beer wort during the fermentation process. The relatively low fat content of brewer's yeast compared to yeast from commercial wet milling ethanol fermentation likely is due to substrate differences (the low fat concentration of barley compared to corn) and differences in the fermentation processes.

Fiber concentration of yeast depends greatly on the method used (Table 2). Although the method of measuring crude fiber (AOAC, 1980) is used for regulatory purposes, results are misleading as several fibrous compounds are solubilized with this procedure, resulting in a large underestimation of fiber content. The neutral detergent fiber (NDF) method of Robertson and Van Soest (1977) results in solubilization of viscous fiber components and recovery of cell wall constituents. Because brewer's yeast contains a considerable amount of protein that becomes viscous when partially hydrolyzed during the NDF procedure, filtration problems and inflated recoveries result in overestimated fiber concentrations (Merchen et al., 1990). For proteinaceous feeds such as brewer's yeast, the method of Prosky et al. (1992) used to measure total dietary fiber (TDF) is most accurate.

Table 2. Fiber composition of brewer's yeast

<table>
<thead>
<tr>
<th>Item</th>
<th>% (DM basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude fiber</td>
<td>0.5</td>
</tr>
<tr>
<td>Total dietary fiber (TDF)</td>
<td>25.1</td>
</tr>
<tr>
<td>Neutral detergent fiber (NDF)</td>
<td>48.2</td>
</tr>
<tr>
<td>Acid detergent fiber (ADF)</td>
<td>6.8</td>
</tr>
<tr>
<td>Acid detergent lignin (ADL)</td>
<td>2.9</td>
</tr>
</tbody>
</table>

1Merchen et al., 1990.

YEAST CELL WALL COMPOSITION

The cell wall of Saccharomyces cerevisiae constitutes approximately 15-30% of the dry weight of the cell and consists primarily of mannosylated proteins, β-glucans, and chitin (N-acetylglucosamine), which are covalently linked. The glucan portion consists of β(1,3)- and β(1,6)-chains. Beta (1,3)-glucans, which form the internal skeletal framework of the cell, are the major structural components and are largely responsible for its mechanical strength. This form of glucan is highly branched and possesses multiple non-reducing ends that function as attachment sites for other components of the cell wall (Kollár et al., 1997). Beta (1,6)-glucans are found primarily outside the skeletal framework and often are linked to cell wall proteins.

Mannose polysaccharides are linked to proteins to form a mannanprotein layer localized at the external surface of the yeast cell wall. Two classes of covalently linked cell wall proteins have been identified. The first class consists of glycosyl phosphatidylinositol proteins that form a complex with β(1,3)- and β(1,6)-chains (Kollár et al., 1997). The second class of cell wall proteins, the protein with internal repeats, are linked directly to β(1,3) glucans. Mannoproteins are strictly regulated in response to changes in external conditions (e.g., heat shock, hypo-osmotic shock, carbon source) and internal changes during the cell division cycle (Horie and Isono, 2001).

While glucans and mannoproteins are main components of the cell wall and found in approximately equal amounts, chitin constitutes only ~1-3% of the cell wall. Although present in small quantities, it is a major component of the primary septum and is involved in the separation of mother and daughter cells, making it essential for cell division (Shaw et al., 1991). The remaining components of yeast, excluding the cell wall, are collectively referred to as yeast cell extract and contain numerous nucleotides, enzymes, vitamins, and minerals.

SELENIUM YEAST

The essential trace element, selenium (Se), has been heavily studied recently as it is thought to play a role in cancer prevention. Selenium is an integral part of the enzyme glutathione peroxidase, which functions to prevent oxidative damage. In the past, sodium selenite (inorganic form) was commonly used as the Se supplement for livestock feeds. However, recent studies have identified organic sources (e.g., selenomethionine) present in plants and selenized yeast as highly digestible alternatives (Yoshida et al., 2002; Gunter et al., 2003). Although growth conditions and yeast strain may influence the proportion of selenocompounds present in selenized yeast, the bulk of the Se is in the form of selenomethionine (Ip et al., 2000; Zheng et al., 2000; Yoshida et al., 2002). Other Se forms present in selenized yeast include selenite and selenoamino acids (selenocystine, selenoanaiphtaniloid, selenocystathionine, Se-methylselenocysteine, Se-adenosyl selenophomocysteine, and γ-glutamyl-Se-methylselenocysteine (Ip et al., 2000). The presence of these highly digestible forms of Se may be partly
responsible for the beneficial effects often observed with selenoyeast supplementation.

**Use of yeasts in companion animals**

Brewer's yeast is used in companion animal foods because it is a high quality protein source rich in B-vitamins, amino acids, and minerals. Inclusion of brewer's yeast (included at 1% of diet) in companion animal diets has been shown to increase (P<0.05) palatability in both dogs (Figure 1; Kennelwood Inc., unpublished data; Ontario Nutri Lab Inc., unpublished data) and cats (Figure 2; Kennelwood Inc., unpublished data) compared to diets containing corn wet milling yeast (included at 1% of diet). In these experiments, consumption ratios of brewer's yeast were 1.9:1 – 2.1:1 compared to corn wet milling yeast. In each experiment, a panel of 20 dogs or cats was used to test food preference with a standard 4-day palatability test. Each day on test, both diets were offered simultaneously for a period of 1 hr. To account for right-left bias, the placement of diets was alternated each day. After the 1 hr feeding period, both diets were removed simultaneously and weighed to calculate intake.

Although few have studied the effects of selenized yeast on companion animal health, one recent experiment reported its beneficial effects on prostate health in aged dogs (Waters et al., 2003). In that

![Figure 1](image1.png)

**Figure 1.** Four-day canine palatability tests. Consumption of dog food including brewer's yeast was greater (P<0.05) than that of a food including corn wet milling yeast (n = 20 dogs in each experiment).

![Figure 2](image2.png)

**Figure 2.** Four-day feline palatability test. Consumption of cat food including brewer's yeast was greater (P<0.05) than that of a food including wet milling yeast (n = 20 cats).
study, 49 elderly (8.5 to 10.5 yr) sexually intact males were randomly assigned to one of five treatments and fed for 7 months: 1) control diet containing 0.3 ppm Se; 2) control diet + 3 µg/kg/d selenomethionine; 3) control diet + 6 µg/kg/d selenomethionine; 4) control diet + 3 µg/kg/d high-Se yeast; or 5) control diet + 6 µg/kg/d high-Se yeast. Although no carcinomas were observed during histopathologic examination, Se supplementation decreased (P<0.001) DNA damage and increased (P=0.04) number of apoptotic cells in prostate epithelia. Because DNA damage and apoptosis may be Se-responsive events that are important regulatory points in prostate carcinogenesis, selenized yeast supplementation may prove to be protective against its development.

Use of yeast components in companion animal foods

‘Functional foods’, ‘nutraceuticals’, and ‘phytochemicals’ are terms commonly used to refer to foods or compounds in foods that possess properties that may benefit the human in ways other than providing nutritive value. Although use of these ingredients began in the human food industry, there is interest in including them in pet foods as well. Many functional ingredients are thought to decrease the incidence of certain disease states or extend the lifespan of pets by possessing antioxidant activity, antimicrobial action, or immuno-enhancing properties. Several components present in yeast may be classified as being functional, including glucomannans, mannans, mannoproteins, β-glucans, and nucleotides.

GLUCOMANNANS

Glucomannans, extracted from the inner cell wall of yeast, may prove to be beneficial in animal foods because of their ability to bind mycotoxins. Mycotoxins are naturally occurring toxic chemicals produced by molds under certain environmental conditions. Sharma and Márquez (2001) tested 12 pet foods commercially available in Mexico for frequency and concentration of aflatoxins. In that experiment, seven aflatoxins and aflatoxicol were detected in most samples, with aflatoxin B1 being present in the highest frequency and concentration. In all contaminated samples, maize was the main ingredient. Research is needed to measure incidence and concentration of mycotoxins in pet foods commercially available in the US, and to determine whether these concentrations are cause for concern. If that is the case, inclusion of glucomannans in pet foods, especially those containing high concentrations of grain, may be prudent. Glucomannans may also play a role in colon cancer prevention because of their antimutagenic and antioxidative activity (Chorvatovicová et al., 1999; Krizková et al., 2001).

MANNANS

Mannans, also referred to as mannan oligosaccharides (MOS), are composed of short chains (attached mainly by α(1,2) and α(1,3) bonds) and long chains (attached mainly by α(1,6) linkages with branches linked by α(1,2) and α(1,3) bonds) (Spring and Dawson, 2000). Mannans have been studied for their ability to agglutinate and interfere with intestinal binding and colonization of harmful microbial species. Numerous E. coli and Salmonella strains possess mannose-specific fimbriae, agglutinate mannans in vitro, and colonize in lower concentrations in animals supplemented with mannans. Fimbrial adhesins specific for mannan residues are referred to as Type-1 adhesins. Mannans aid in the resistance of pathogenic colonization by acting as receptor analogues for Type-1 fimbriae and decrease the number of available binding sites (Oyofo et al., 1989).

Mannans are capable of modulating the immune system and influencing microbial populations in the gut. Mannans (Bio-Mos®) have been reported to increase (P=0.14) serum IgA concentrations in dogs (2.33 vs 1.93 g/L, Swanson et al., 2002a). In adult dogs, mannan oligosaccharides as Bio-Mos® beneficially altered microbial ecology by increasing (P=0.13) lactobacilli populations (9.16 vs 8.48 log10 CFU/g fecal dry matter) and decreasing (P=0.05) total aerobe populations (7.68 vs 8.67 log10 CFU/g fecal dry matter, Swanson et al., 2002a).

MANNOPROTEINS

Recent experiments have suggested that mannoproteins could be promising vaccine candidates for individuals with compromised T-cell function (e.g., AIDS, lymphoma). Mansour et al. (2002) determined that mannoproteins are ligands for the macrophage mannose receptor, which serves as a link between innate and adaptive immunity (Sallusto et al., 1995).
Mannoproteins also have been shown to elicit delayed-type hypersensitivity reactions and induce production of cytokines important in decreasing fungal pathogens (Chaka et al., 1997; Pietrella et al., 2001).

**ß-GLUCANS**

Although much of the ß-glucan research has focused on oat bran, experiments using ß-glucans derived from yeast have resulted in similar findings. Of all the beneficial properties that have been reported, the lipid-lowering effect of ß-glucans probably has been the most popular. An experiment using free-living, obese, hypercholesterolemic men demonstrated that yeast-derived ß-glucans were well tolerated and decreased (P<0.05) blood total cholesterol concentrations similar to the effect of oat products (Nicolosi et al., 1999). Yeast-derived ß-glucans also appear to possess antimicrobial and antitumor properties by enhancing immune function. The binding of ß-glucan to its receptor present on macrophages results in phagocytosis, respiratory bursts, and secretion of TNF-α (Chen and Hasumi, 1993; Lee et al., 2001). Finally, ß-glucans are readily fermented in the large bowel and serve as a fuel source for microbial populations.

**NUCLEOTIDES**

In contrast to the components listed above, nucleotides are present in yeast extract rather than cell wall. Although endogenously produced by the body, dietary nucleotides may be essential in certain life stages or in certain health conditions (e.g., neonates, immune-compromised) (Sánchez-Pozo and Gil, 2002). In addition to stimulating the development of the small intestine (Bueno et al., 1994) and liver (Sánchez-Pozo et al., 1998), exogenous nucleotides have been shown to enhance immune function by increasing production of immunoglobulins, improving response to vaccines, and increasing tolerance to dietary antigens (Maldonado et al., 2001). Because of their importance in neonatal nutrition, the inclusion of nucleotides in human infant formulas is under investigation (Cordle et al., 2002; Ostrom et al., 2002).

**IN VITRO RESEARCH ON YEAST COMPOUNDS**

Limited research has been performed testing the effects of yeast or yeast fractions on dog and cat health, with mannans being the only fraction studied to any extent. Vickers et al. (2001) used canine fecal inoculum to determine the fermentability characteristics of mannan oligosaccharides. In that experiment, moderate concentrations of total short-chain fatty acids were produced after 6 (0.49 mmol/g of organic matter), 12 (1.45 mmol), and 24 hrs (2.40 mmol/g) of in vitro fermentation. The microbial species responsible for mannan oligosaccharide breakdown were not determined in this experiment.

Hussein and Healy (2001) also performed an in vitro experiment using canine and feline fecal inoculum to determine fermentability of mannan oligosaccharide in Bio-Mos® (Alltech Inc.). Differences were not observed in fermentability between dog and cat fecal inoculum. By examining dry matter and organic matter disappearance, it appeared that mannan oligosaccharide was highly fermented. Dry matter disappearance after 6, 12, 18, and 24 hrs of in vitro fermentation was 54.3, 57.9, 60.7, and 61.3%, respectively. Organic matter disappearance was similar to that of dry matter (56.8, 60.7, 63.7, and 64.1% after 6, 12, 18, and 24 hrs of fermentation). Dry matter and organic matter disappearance do not always reflect microbial fermentation due to the disappearance of soluble carbohydrates present in the substrates that are not retained during filtering. Although soluble carbohydrates are available for fermentation, gravimetric methods cannot determine the proportion used by the microbes as an energy source. Therefore, the measurement of dry matter and organic matter disappearance is not as accurate as the measurement of the fermentation end-products (i.e., short-chain fatty acids and gas), which is a direct measurement of fermentation. Concentrations of total short-chain fatty acids, acetate, and propionate increased linearly over time. Moderate concentrations of total short-chain fatty acids (10.1, 26.8, 36.7, and 49.7 mM) were produced after 6, 12, 18, and 24 hrs. In comparison to total short-chain fatty acids, lactate concentrations were fairly high (7.7, 8.7, 7.6, and 5.9 mM), suggesting fermentation by a lactate-producing species (e.g., lactobacilli, bifidobacteria). In agreement with the work of Vickers et al. (2001), these data suggest that mannan oligosaccharide is moderately fermentable by canine and feline microflora. The lactate produced during fermentation suggests that lactate-producing species are able to utilize mannan oligosaccharide, possibly by acting as a prebiotic for these species.
In vitro data from our laboratory indicated that mannan oligosaccharide was highly fermentable by canine fecal inoculum (Swanson, unpublished data). After 0, 4, 12, and 24 hr of in vitro fermentation, organic matter disappearance was 38.1, 40.5, 41.5, and 60.4%, respectively. The relatively high organic matter disappearance at 0 hrs was not due to microbial fermentation, but rather to the soluble carbohydrates present in mannan oligosaccharide not being retained during filtering. After 4, 12, and 24 hrs of fermentation, corrected total short-chain fatty acid concentrations were 1.18, 2.71, and 4.60 mmol/g organic matter, respectively. These short-chain fatty acid concentrations were approximately twice as high as those reported by Vickers et al. (2001) at the 12 and 24 hr time points. In agreement with short-chain fatty acid data, gas was produced in relatively high amounts (corrected gas values were 17.4, 58.3, and 100.9 mL/g organic matter after 4, 12, and 24 hrs of fermentation).

CANINE RESEARCH ON YEAST COMPONENTS

O’Carra (1997) performed two experiments examining mannan oligosaccharide in Bio-Mos® (Alltech Inc.) and its effects on immune function in dogs. In the first experiment, adult beagles were fed diets containing 0, 1, 2, or 4 g Bio-Mos®/kg diet. Changes in plasma protein and IgG measurements were not observed after 15 or 31 days of supplementation. In the second experiment, Border collie pups were fed diets containing 0 or 2 g Bio-Mos®/kg. After a 7-day adaptation period, a vaccination protocol was initiated. All dogs were vaccinated against parvovirus, leptospirosis, adenovirus, and distemper. Vaccine boosters were applied on day 21 for leptospirosis and on day 35 for parvovirus. Blood characteristics were measured over a 9-wk period. No changes were observed in weight gain, lysozyme activity, plasma protein concentration, or plasma IgG concentration. Neutrophil activity was numerically increased in pups fed the diet containing Bio-Mos® after vaccination [approximately 18 vs 14 Nitroblue tetrazolium (NBT)+ cells/slide]. However, due to low animal numbers (n = 3/group), statistical significance was not reached.

Using adult ileal cannulated dogs, Strickling et al. (2000) compared a control diet to those containing 5 g oligosaccharide/kg diet, one of which was Bio-Mos®. Researchers measured ileal and total tract nutrient digestibilities, microbial populations, ileal pH, ammonia and short-chain fatty acid concentrations, blood glucose, and fecal consistency. Besides minor changes in short-chain fatty acid concentrations, the only relevant finding was a decrease (P = 0.07) in Clostridium perfringens populations in dogs fed Bio-Mos® (4.48 log10 CFU/g) vs dogs fed xylooligosaccharides (5.16 log10 CFU/g) or oligofructose (4.74 log10 CFU/g). Because clostridia species do not possess mannose-specific fimbriae, another mechanism is likely occurring. The lack of any significant findings may be due to the low dose of prebiotics consumed (only ~1.3 g/d) or to the use of soybean meal in the control diet, which supplied an estimated 10 g/kg of naturally occurring oligosaccharides, mainly galactooligosaccharides. Any beneficial effects resulting from Bio-Mos® consumption may have been masked by the presence of these naturally occurring oligosaccharides.

Zentek et al. (2002) used 4 dogs in a 4 x 4 Latin square design to determine the effects of mannan oligosaccharide (Bio-Mos®), transgalactosylated oligosaccharides, lactose, and lactulose on fecal characteristics, total tract digestibility, and concentrations of microbial end-products in feces and urine. Carbohydrate supplements were administered at a rate of 1 g/kg BW/d. Mannan oligosaccharide supplementation decreased (P<0.05) fecal pH (6.6 vs 6.9), fecal ammonia excretion (78.4 vs 116 µmol/g feces), and apparent dry matter (81.9 vs 85.0%), crude protein (79.8 vs 82.5%), and nitrogen-free extract (83.1 vs 94.8%) digestibilities. By decreasing fecal pH and ammonia, mannan oligosaccharide supplementation appeared to improve indices of colonic health. However, the decreases observed in apparent nutrient digestibilities resulting from mannan oligosaccharide supplementation would increase fecal quantity and the cost of feeding the animal. The dose of carbohydrate supplements fed in this experiment (1 g/kg BW/d) was very high. Smaller doses of mannan oligosaccharide may not have such negative effects on nutrient digestibility.

Using ileal cannulated adult dogs, Swanson et al. (2002a) examined the effects of supplemental mannan oligosaccharide (Bio-Mos®) and (or) fructooligosaccharides (FOS) on colonic microbial populations, local and systemic immune function, fecal protein catabolite concentrations, and ileal and total tract nutrient digestibilities. A 4 x 4 Latin square design with 14-day periods was used. Twice daily, dogs were offered 200 g of dry, extruded, kibble diet and given the following treatments orally via gelatin capsules: 1) control (no supplemental MOS
or FOS), 2) 1 g FOS, 3) 1 g MOS, or 4) 1 g FOS + 1 g MOS. Mannan oligosaccharide supplementation beneficially influenced microbial populations, decreasing (P=0.05) total aerobe (7.68 vs 8.67 log_{10} CFU/g fecal dry matter) and tending to increase (P=0.13) Lactobacillus populations (9.16 vs 8.48 log_{10} CFU/g fecal dry matter). Mannan oligosaccharide also increased serum IgA concentrations (2.33 vs 1.93 g/L, P=0.14) and lymphocyte numbers (20.4 vs 15.6% of total white blood cells, P<0.05). A tendency for decreased ileal dry matter (55.0 vs 67.7%, P=0.15) and organic matter (63.6 vs 74.1%, P=0.15) digestibility also was observed from Bio-Mos® supplementation. The combination of FOS and MOS supplementation enhanced immune characteristics, increasing ileal IgA concentrations on a dry matter basis (4.90 vs 3.40 mg/g ileal dry matter, P=0.06) and crude protein basis (12.22 vs 8.22 mg/g ileal crude protein, P=0.05). Supplementation of FOS + MOS also decreased (P<0.05) total fecal indole and phenol concentrations (1.54 vs 3.03 µmol/g fecal dry matter), compounds partially responsible for fecal odor and detrimental to intestinal health. This experiment was performed using healthy adult dogs, which would not be at the highest risk for intestinal irregularities. It is likely that the health benefits of feeding mannan oligosaccharide alone, or in combination with FOS, would be more beneficial to populations of elderly dogs, young weanling puppies, or stressed animals.

In a follow-up study, Swanson et al. (2002b) supplemented ileal cannulated dogs with either 1 g sucrose (placebo) or 2 g FOS plus 1 g Bio-Mos®. Fecal, ileal, and blood samples were collected at the end of each 14-day period to measure microbial populations and immune characteristics. Supplementation of FOS plus MOS increased (P<0.05) fecal bifidobacteria (10.04 vs 9.42 log_{10} CFU/g fecal dry matter) and lactobacilli concentrations in feces (9.75 vs 8.24 log_{10} CFU/g fecal dry matter) and ileal effluent (8.66 vs 7.55 log_{10} CFU/g ileal dry matter). Dogs fed FOS plus MOS also tended to have lower (P=0.08) blood neutrophils (62.99 vs 66.13 % of total white blood cells; 6.40 vs 7.22 x 10^1 cells/µL) and greater (P=0.06) blood lymphocytes (19.95 vs 17.29 % of total white blood cells) vs placebo. Serum, fecal, and ileal immunoglobulin concentrations were unchanged (P>0.05) by treatment. Supplementation of FOS plus MOS beneficially influenced indices of gut health by improving ileal and fecal microbial ecology and altered immune function by causing a shift in blood immune cells.

Grieshop et al. (2004) tested the effects of chicory and (or) mannan oligosaccharide on nutritional and immunological characteristics of geriatric dogs. After a 4-wk baseline period, 34 senior dogs (beagles: 9-11 yr old; pointers: 8-11 yr old) were randomly allotted to one of four treatments: 1) control (no chicory or Bio-Mos®); 2) 1% chicory; 3) 1% Bio-Mos®; or 4) 1% chicory + 1% Bio-Mos®. Dogs remained on treatment for 4 wks. Increased (P=0.07) food intake by dogs fed chicory + MOS and MOS alone resulted in increased (P<0.05) wet fecal output. Dry matter, organic matter, and crude protein digestibilities were unchanged due to treatment. Supplementation of Bio-Mos® increased (P<0.05) fecal bifidobacteria populations and decreased (P<0.05) fecal E. coli populations compared to control. Supplementation of chicory + Bio-Mos® tended to increase (P<0.10) neutrophil concentrations, while Bio-Mos® (P=0.06) and chicory + Bio-Mos® (P<0.05) decreased lymphocyte concentrations. Finally, prebiotic supplementation altered proportions of lymphocytes expressing CD4 and CD8 cell surface markers. Chicory + Bio-Mos® supplementation decreased (P=0.07) CD8-specific lymphocytes. Results of this experiment support findings from previous experiments that in addition to altering gut microbial ecology, Bio-Mos® supplementation may affect immune status.

**ACTIVE COMPONENTS IN YEAST COMPONENTS**

Our laboratory has analyzed several commercially available sources of mannan oligosaccharides and found considerable differences in crude protein, fat, total dietary fiber, and monosaccharide concentrations (Table 3). Most of the monosaccharides are present as part of polysaccharides rather than as free sugars. Source B was unique in that it contained a considerable amount of galactose in addition to glucose and mannose. The presence of galactose in that source may suggest that guar gum or locust bean gum, which contain galactomannans, are also present in this source of MOS. Although marketed as a source of MOS, these products are very complex and also contain glucans, mannoproteins, phosphate, and several other compounds that apparently are not excluded in the crude extraction process. Because the composition of MOS is complex, the components that result in beneficial effects are not known. Although the mannan portion of MOS is generally thought to be responsible for the pathogenic resistance effect...
by acting as a receptor analog for Type-1 fimbrial adhesions present on species such as *E. coli* and *Salmonella*, it is possible that a different fraction present in MOS is responsible for its effects on immune function. For example, mannoproteins and β-glucans taken from yeast cell walls have been reported to enhance immunity. Therefore, more research is needed in order to determine whether bioactive peptides, β-glucans, mannans, or unknown factors present in MOS are responsible for the immune responses observed as a result of their supplementation.

Table 3. Chemical composition of several mannan oligosaccharide sources.

<table>
<thead>
<tr>
<th>Item</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>91.1</td>
<td>92.1</td>
<td>93.4</td>
<td>94.4</td>
<td>96.5</td>
</tr>
<tr>
<td>OM, %</td>
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<td>82.2</td>
<td>93.0</td>
<td>93.5</td>
<td>98.1</td>
</tr>
<tr>
<td>CP, %</td>
<td>33.7</td>
<td>39.0</td>
<td>34.4</td>
<td>42.2</td>
<td>42.9</td>
</tr>
<tr>
<td>TDF, %</td>
<td>37.0</td>
<td>21.4</td>
<td>40.8</td>
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<td>NA</td>
</tr>
<tr>
<td>Fat, %</td>
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<td>6.1</td>
<td>7.7</td>
<td>6.6</td>
<td>12.1</td>
</tr>
<tr>
<td>Glucose, mg/g</td>
<td>274.0</td>
<td>214.4</td>
<td>341.6</td>
<td>345.5</td>
<td>188.4</td>
</tr>
<tr>
<td>Mannose, mg/g</td>
<td>119.3</td>
<td>58.6</td>
<td>99.7</td>
<td>90.7</td>
<td>144.0</td>
</tr>
<tr>
<td>Galactose, mg/g</td>
<td>ND</td>
<td>35.9</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Free glucose, mg/g</td>
<td>1.8</td>
<td>2.0</td>
<td>1.6</td>
<td>1.8</td>
<td>ND</td>
</tr>
<tr>
<td>Free mannose, mg/g</td>
<td>ND</td>
<td>TR</td>
<td>ND</td>
<td>0.1</td>
<td>ND</td>
</tr>
<tr>
<td>Free galactose, mg/g</td>
<td>ND</td>
<td>1.1</td>
<td>TR</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

1% of dry matter;
2ND = not detected.
3TR = trace.
4NA = not analyzed.

Conclusions

Relatively little research has been performed with companion animal species, but the limited data suggest that inclusion of brewer's yeast or yeast components in pet foods may support gut health. Although brewer's yeast often is included for palatability enhancement, its functional properties may be an even more important reason for its inclusion in pet foods. From the limited number of experiments testing mannan oligosaccharide, it appears that it has beneficial effects on indices associated with gut health. Mannan oligosaccharide supplementation has resulted in improved ileal and fecal microbial ecology and enhanced immune status. Because glucomannans are able to bind mycotoxins, their presence in pet foods also may be beneficial. Finally, yeast-derived β-glucans, mannoproteins, and nucleotides require further testing to determine their role, if any, in companion animal nutrition and health.

References


The expanding pet food industry: where are the opportunities?

TIM PHILLIPS

PETFOOD INDUSTRY Magazine, Watt Publishing Co., Mt. Morris, Illinois, USA

Introduction

The pet food market is full of dynamic and diverse opportunities, according to a report by Lisansky (2003). The global market has boomed with new products, new presentations and new focus on pet population sectors. There is solid and steady growth in pet foods, particularly in dry cat and dog food areas. Pet ownership has also increased, and improving pet nutrition through consumption of commercial pet foods and treats is on the rise.

Geographic expansion is also booming with the areas of southern Europe becoming more affluent and converting to manufactured pet food, opening up manufacturing and distribution opportunities for local manufacture.

Pet food has become more targeted on specific life stages. For example, there are foods for kittens, adults, queens and seniors. Packaging has become more convenient and more expensive with single-meal cans and pouches replacing large cans.

Pet food now comes in several forms. There are homogeneous mixtures, chunks, some with jelly and some with gravy. Dry pet food is no longer the standard issue kibble left for pets between meals. It is now available over a much wider range of flavors and prices. Up-market dry pet food, first sold in veterinary surgeries and then in specialist stores, has now moved into mainstream supermarkets, suggesting much more is being sold.

Pet food has the advantage over human food in that people often love their pets more, and are more willing to spend extra money to keep them happy and healthy. Many owners do not buy on price, but on pet acceptability, giving ingredient manufacturers superb opportunities to enter the market with better products.

It seems likely that in the near future, ingredients like omega-3 fatty acids and other ‘healthy’ human food ingredients will become more common in pet food. Probiotics and prebiotics, separately and combined as synbiotics, now popular for humans and in animal feed, are likely to become components of pet food. Many botanicals, known for their effects on humans, are also going to become ingredients of new ‘healthy’ pet food.

Specialist pet foods for specific health conditions already exist and should become more popular as pets live longer and humans spend more to protect them. Low protein, low ash and easily digestible pet foods are useful for owners wanting to keep pets healthy.

Major pet food producers are becoming more specific in their requirements and are vigorously communicating their needs to ingredient companies. They want fewer commodity ingredients and more specialty ingredients for both existing and new products. They are willing to pay more for the ‘right’ ingredients since they can pass on the cost to consumers who will buy the ‘right’ products. Such specialty ingredients should provide improved technical functionality, supply health benefits or perhaps both. The growth in dry foods in Europe and North America, although threatening to erode the market for wet food to some extent, may also open up opportunities for new ingredients including ‘healthy’ ingredients. For example, hydrocolloid companies who may lose some market in wet pet foods could take the opportunity to regain market in dry pet food, perhaps by attempting to develop health ingredients based on soluble fiber or to use hydrocolloids as carriers for healthy ingredients.
In the food industry, the development of the 'functional food' market continues, with food producers making health claims for a variety of products. With the increasing consumer awareness of health benefits delivered in food and beverage products, it is likely that the trend for 'healthier' foods will also have an impact on the consumer's choice of pet foods. Pet owners are likely to pay particular attention to the dental, digestive, circulatory and immune health of their pets in order to improve longevity and quality of life.

Pet food ingredient types

Pet food ingredients fall into three principal categories: commodities, semi-commodities, and specialties. The commodity ingredients, many also used in food manufacture, include starches and fats, bulk proteins, fibers and other bulking agents. Marketing strategies for these products rely on providing relatively low prices while maintaining product standards, specifications and safety. The opportunities for expanding markets include moving geographically into areas of increasing pet food use as well as taking market share from competitors with a better offering.

Semi-commodity items include flavorings like protein hydrolysates, texturizers such as gluten and gums, and nutritional items like vitamins, minerals and premixes. Marketing strategies are oriented towards performance and value for money combined with reliability and customer services. Many opportunities exist for market expansion of semi-commodities by creating points of differentiation with competitors; this requires both product development and customer support. The higher margins available can justify the investment and effort.

Specialty ingredients, including specialty fats and oils, high-quality palatants and flavors, custom premixes, fermentation and cheese products, have the best margins and the lowest volumes. Specialties are what most companies strive to sell, as they have high barriers to entry. However, developing and proving the value of specialties can be a lengthy and expensive task; the strategy for market expansion should be carefully planned and costed at the start.

Exploring new markets

GETTING STARTED

Experience suggests that there is hard homework to do that requires a lot of data and analysis. Companies can carry out these investigations themselves and usually do an excellent job, but the work is time consuming and often must be done by a deadline. Consultants can provide the extra resources required to ensure a thorough and timely review. They also can investigate new markets without alerting competitors or potential customers. This anonymity is a big advantage. Lastly, consultants have an objective viewpoint and can tell companies what they need to know rather than what a senior executive wants to hear.

A systematic and scientific approach should be used to explore the possibilities of new markets. It is wise to determine the nature of the opportunity before taking action. One should find the most cost-effective approach and select ways of approaching it that reduce the business risk while simultaneously increasing strategic choices.

First, research should be used to determine the current market situation. Not only is the market volume and value important, the past and future trends should be identified in the context of the economic and regulatory environments. Experts should be consulted from as many sources as possible – from customers, suppliers and competitors to get as close to the 'truth' of the market as possible.

The most important aspects of market analysis are identifying the key factors for success and the barriers to entry. This applies whether one is looking for a better strategy for market penetration, to expand geographically or to launch new products. Also useful is a detailed ‘SWOT’ analysis of one’s company – strengths, weaknesses, opportunities and threats. Once the data are analyzed and the opinions considered, then usually it is possible to generate conclusions and recommendations for the best options.

STRATEGY FOR GROWTH BY GEOGRAPHIC EXPANSION

Geographic expansion is one of the first things companies think about when considering expansion. Most companies have a geographic area with which they are familiar. They understand the subtle dynamic of doing business there – how many times new customers need to be seen before they buy, what kind of service customers want and are prepared to pay for, what are the key success factors in making a sale. Companies often think that going into a new geographic region will be an extension of their previous way of doing business. This is almost never true.
In the US, companies, customers and suppliers want to talk about the price, the specification, the delivery criteria, the guarantees and occasionally about customer service, but are wary of having to pay for it. They will often trade loyalty for price, as in strategic supplier alliances, where qualified suppliers get minimum assured orders in exchange for reduced prices. In the US, especially in the ingredients business, price remains overwhelmingly the most important criterion on which supplier offers are judged.

In the EU, the situation can be quite different. Products are sold on price, but other factors can contribute significantly to making sales. One example from well outside the pet food market was a company making road-paving equipment in the US and in the EU. Machines in both regions were sold on price, but in the US the machines had an unfinished and economical appearance, whereas in the EU all the metal had to be smoothed and carefully painted before a customer would consider buying it. The company’s US staff could not understand why machines made to the US specification would not suffice for the EU market, nor could they imagine themselves selling machines ‘tarted-up’ like those made in Europe. The cultures were different and irreconcilable with one model. To be successful, the company had to adapt its production and sales methods to suit two different groups of customers.

The EU will tolerate higher ingredient prices and higher prices for finished products than can be obtained in the US, if the performance of the product will attract more sales. This represents an opportunity for US companies to earn higher profits in the EU, but they have to tailor the marketing and sales approach to fit these customers.

Conversely, the US remains the land of opportunity for many EU companies. However, the requirements of US customers can be very different from those in the EU.

Why should this be true? The following observations are perhaps over-stated generalizations, but they are made to illustrate that differences exist and should be considered when planning market expansion.

In the food and pet food businesses, one important difference between the US and the EU is the strength of the retailer. In the US, it is commonly accepted that the retailer is a ‘realtor’ renting shelf space to manufacturers to display their products. The retailer makes a small margin and may have relatively little interest in whether the product sells or not. Displays and stock management can be left to the manufacturers. Even the production and management of ‘store brands’ can be contracted out and handled entirely by a contract supplier.

Because the US is so big and heterogeneous, most retailers in the US are regional, while many food and pet food companies are national or multinational. The two groups of companies are different in size and in how they perceive and manage the ultimate customers. Retailers are at the ‘point-of-sale’ with customers but their economic success may not depend very much on what those customers think.

By contrast, in the EU where the countries are much smaller and within each country much more culturally homogeneous, retailers have become national and larger than in the US. They compete for market share within their own countries; they organize the logistics of national supply and distribution. They make money, less from renting space, and more from selling products. Successful EU retailers earn a much higher return on investment than retailers in the US.

Consequently, EU retailers make greater efforts to cater to consumers and will dictate their requirements to food manufacturers, rather than allow the manufacturers to determine what products are sold in their stores. There is less shelf space available, and that space must be used with maximal efficiency for the company to make a profit.

INVEST IN EXPANSION

There are many different ways for a business to expand—all businesses are different and most have different objectives. The optimal strategy depends not only on the type of product, but also on the dynamics of the target market. Successful geographic market development relies on good local knowledge in terms of consumer preferences, regulation and marketing practices as well as an appraisal of local competition and the barriers to entry.

Many companies fail to take full advantage of an existing or a new market because of inadequate groundwork and preparation. Companies must invest more time, money and resources in order to carry out a thorough investigation of opportunities to expand their businesses.

INDUSTRY CHANGE

Globalization means accelerated growth for the pet food industry. Factors that are leading to globalization include:
The expanding pet food industry: where are the opportunities

- **Falling trade barriers.** As barriers fall, pet food companies can use more efficient plants to serve groups of countries vs building a plant in each country.

- **Homogenized preferences.** Pet owners are becoming similar in their pet food wants and needs. Increasingly, they are exposed to the same media messages (news, entertainment, sports, advertising).

- **Big technology investments.** Pet food product development is becoming more sophisticated. Manufacturing process research is also becoming more important.

- **Easier movement.** It is getting easier, faster and cheaper to move information, money and people around the world. Thus, the cost of adopting a global strategy is lower.

What does globalization mean to the pet food industry? Pet food companies are likely to become larger, more international and more sophisticated in terms of formulation, processing and packaging. Globalization means consolidation, expansion and accelerated market development.

### Growth strategies

It would be easy to look at the pet food industry today and say, “If I’m not Nestlé or Mars, I might as well give up.” Large, dominant firms seem to have all the marketing clout, financial leverage, production economies of scale, channel power, R&D capability and ability to respond to new opportunities, leaving smaller competitors with few options but to wait for the inevitable hostile takeover or market oblivion. But such a defeatist attitude is premature, to say the least. Anti-trust action aside, the fact is that the top five firms (counting Nestlé and Ralston Purina as a single company) accounted for a little more than half the market share in the US. Market dominance has clearly not occurred yet (Table 1).

A look at the future, however, may be more ominous. Of thirteen major new product introductions in 2000 and 2001, ten (or 77%) came from one of these top five firms. This statistic, while far from conclusive, nevertheless suggests that innovation is occurring at a more rapid rate in deeper-pocketed companies, and that increasing market dominance by larger firms may be just around the corner.

<table>
<thead>
<tr>
<th>Company</th>
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<td>7.9</td>
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<tr>
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<td>3. Iams Co., The</td>
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<td>0.0</td>
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</tr>
<tr>
<td>11. Heinz Co., HJ</td>
<td>1.2</td>
<td>0.04</td>
</tr>
<tr>
<td>12. Royal Canin SA</td>
<td>0.56</td>
<td>0.0</td>
</tr>
<tr>
<td>13. Private label</td>
<td>3.01</td>
<td>3.2</td>
</tr>
</tbody>
</table>

*Euromonitor estimates that global pet food sales (retail, except Doane) were US$32 billion in 2002.

*Euromonitor and company sources.

Does it have to be this way? Not necessarily. A trend towards greater size is not inevitable, and certainly does not spell oblivion for smaller competitors.

When companies expand, they require more resources and capabilities to meet the needs of new markets, new product lines, and greater scope of operations. For example in the international arena, one of the biggest barriers to a firm’s growth can be a lack of managers with the appropriate training and experience. In fact, research by Nitsch (2003) suggests that the availability of managerial resources is the strongest determinant of a company’s decision on whether to establish a joint venture or a wholly owned subsidiary in a new location. A lack of managers is linked to a preference for joint venturing, to a significantly greater degree than a lack of financial or technical resources. Nevertheless, a lack of technology or money can also be a motivation for seeking a partnership instead of going it alone.

Nitsche’s research supports a view of strategic management that is based on resource availability. A firm, confronted with market opportunities, should decide to pursue those that it can execute effectively, not those which generically seem to be the most attractive. It is more important to do things right than to do the right things. If this is true, then a firm presented with strategic alternatives has two choices when it comes to assembling the resources necessary to implement them: either develop or supply the resources internally, or obtain them from outside the firm.
THE POTENTIAL OF ALLIANCES

All forms of business activity, with the exception of wholly owned subsidiaries, can be broadly called strategic alliances. The partners may be manufacturer and distributor, or licensor and licensee, in which case there is a contractual alliance. Alliances can also entail an exchange or pooling of equity capital, used for the creation of a new venture or the purchase of an existing business in the foreign country. Equity joint ventures are thus a special case of strategic alliances. In the pet food context, all such relationships are means for the participants to gain access to resources that they do not possess themselves, as a means of obtaining a competitive advantage.

If the managers in a firm lack the needed market knowledge, for example, a joint venture with a local partner, licensing to a local manufacturer, or exporting to a local distributor may be a wiser choice than attempting to go it alone. Similarly, a need to upgrade the firm’s skills technologically might lead to a decision to create a joint venture with a partner that has the desired capabilities, and from whom the firm can learn. The reverse is also possible, that is, a local company can be a licensee to a foreign firm, or become a local distributor for someone else’s product. Some companies are both licensors of some of their own products, and licensees for those of others.

Alliances can thus represent an opportunity for a relatively small player in an industry to ‘punch above its weight’ in the competitive arena. Technology lacking? License someone else’s product to earn incremental profits or fill a market niche. Not big enough to get maximum scale economies? Have your product produced by a larger competitor with excess capacity. Can’t afford the R&D to do what you need? Pool resources with others to amass the research bench strength necessary.

Obviously, there is no free lunch. Alliances, especially equity joint ventures, represent a collaboration out of which all partners must be able to gain something. This is one of the key things to remember in the search for an appropriate alliance partner. It can be useful to think not in terms of who is the ideal partner, but how you can be a better partner yourself.

Alliances can also be challenging to manage well. The sources of potential conflict are endless, ranging from questions about the scope of activity covered by the alliance, to disputes over distribution of the gains, staffing issues, managerial authority, strategic goal misalignment, and even personality differences between the principals. The management costs, both in terms of actual expenditures and in terms of management time and focus, can be enormous. Nevertheless, alliances of all kinds are increasing in popularity across all industries. This is because the potential benefits can more than offset the costs. The opportunity to form an alliance can often make the difference between taking advantage of a market opportunity and not being able to enter the market at all.

Skill in forming and managing alliances in a fast-moving industry like pet food can also determine the difference between companies that are able to survive and prosper, and those which fade away into irrelevance.

Noteworthy trends

The global market for pet food and pet care products rose 2.6% to reach US$46 billion in 2002 (retail sales), recording a stronger increase compared to the two previous years (Crossley, 2003). Sales growth was depressed in 2000 and 2001, partly as a result of weak currencies against the US dollar. In volume terms, the market also recorded growth reaching 16.7 million tons.

MAJOR MARKET SALES

North America is the largest region, with 41% of global market value in 2002, 39% coming from the US. The US remains by far the largest national market in terms of total value sales, and has by no means reached saturation. While over 90% of cats are currently being fed prepared food, the penetration levels of prepared dog food continue to be lower, standing at less than 80% in 2002. Consequently, there remains strong possibility for growth, in particular for dog food. A continuing rise in the cat population ensures a growing consumer base in this sector. Growth is moreover a result of consumers moving to premium food and spending money on non-essential products such as treats or pet care products. Total market value rose by nearly 5% in the US during 2002 (Figures 1 and 2).

Western Europe accounts for a further 30.5%. Similarly to North America, a rising pet population and growing expenditure per pet resulted in strong value growth in the region.
Japan, the next largest market, represents 13%, although this market is currently stagnating as a result of the declining pet population and weak economic environment. Pets purchased during the ‘pet boom’ years of the 1990s gradually died off, and the weak economic environment resulted in fewer consumers being willing to replace their pets.

Mexico, which ranks ninth in terms of total value sales, recorded one of the strongest growth rates. Value growth of over 25% in 2002, and nearly 140% from 1998 to 2002. Rapidly rising penetration levels of industrially prepared dog and cat food, partly a result of rising disposable incomes, and supported by consumer education on pet nutrition, are the main reasons for particularly strong growth in this now major market.

Although China’s value sales seem marginal in comparison with major markets of Western Europe or Latin America, the country’s sales increased rapidly and its growth potential is indeed remarkable. In 2002, value rose by nearly 13%, as rising disposable incomes and a gradually improving retail infrastructure enable more and more pet owners to purchase prepared food as well as pet care products.
GROWTH STIMULANTS

Manufacturers lead growth in mature markets

In mature markets of North America, Western Europe and Japan, manufacturers lead market growth, often through new product launches. As penetration levels of prepared dog and cat food are high and even close to saturation in some markets, product innovation is of paramount importance to expansion, encouraging consumers to move to higher value products utilizing advanced formulations for food, health care and hygiene products. Product segmentation strategies also stimulate growth, via premium brands for various life stages, lifestyles and breeds of pets.

‘Humanization’ of pets

The ongoing trend towards ‘humanization’ of pets has also proven instrumental in sustaining growth in even the most saturated of markets, with consumers inclined to spend excessively on products echoing human tastes. This was most evident in the growth of gourmet flavors of pet food, particularly for cats, mirroring human taste in cuisine, but was also evident in the growth of treats, gifts and other extravagant luxury items purchased for animals by their owners. Humanization is also increasingly prevalent in emerging markets, as evident by the growing popularity of nonessential pet care products in countries such as China and South Korea.

Improvements in distribution add value to global sales

In the major markets of North America, Western Europe and Japan, growth of the pet superstore made pet accessories and – most importantly – premium and superpremium dry cat and dog food easily accessible to a mainstream audience. This trend was compounded with the introduction of the superpremium brand Iams into supermarkets in the US and parts of Western Europe. Meanwhile, the growth of supermarkets in major emerging markets such as China and Brazil served to increase usage of prepared pet food.

Increase in the pet population ensures customer base growth

A worldwide increase in the pet population – particularly in cats and dogs – ensures that the customer base will continue to grow, resulting in strong growth in volume terms. Pet ownership is expected to continue to rise in the foreseeable future, as urbanization and hectic lifestyles often result in people living on their own, turning to pets as companions.

Rising pet ownership levels in emerging markets

In emerging markets, growth was also a result of rising pet ownership levels. Value growth was, however, not dependent on consumers moving to premium varieties, but a result of rising penetration levels of industrially prepared dog and cat food. Rising disposable incomes, as well as efforts by manufacturers and trade associations to educate consumers on pet nutrition, play a major role in the increasing acceptance of prepared food in emerging markets.

Growth surge in premium and superpremium dry food

Growth in most mature markets was underpinned by the rise of higher-priced dry premium and superpremium pet foods, particularly in the core dog and cat food sectors. A trend towards such products was noticeable in North America, Western Europe as well as Japan. It was partly a result of manufacturers’ product innovation being concentrated in the premium end of the market—products which in turn were very well received by consumers who are increasingly concerned about pet health and well-being.

Economy and mid-priced brands maintain a large customer base

Although premium dog and cat food enjoyed more dynamic growth, economy and mid-priced brands continue to have a large customer base. For many cost-conscious consumers, mid-priced products in particular are regarded as offering sufficiently high quality to ensure adequate nutrition for their pets. Moreover, many mid-priced products now also incorporate the life stage/lifestyle concept previously only available within the premium segment. In most major markets, in volume terms, sales of mid-priced dog and cat food continue to be higher than those of premium food.

Wet vs dry

In most developed markets, dry dog and cat food enjoyed more dynamic growth compared to wet
The expanding pet food industry: where are the opportunities

varieties, due to health and convenience reasons. The trend towards premium, life stage- and lifestyle-specific products also led to the increasing popularity of dry food. As dry products can be bought in bulk, are relatively easy to store and do not emanate unpleasant odors, dry food enjoyed stronger growth compared to wet food for both dogs and cats. Dry dog and cat food also dominate in emerging markets, albeit for different reasons. As the unit prices for wet food are very high, most pet owners who are willing and able to purchase prepared food opt for dry products. Wet food has premium status in emerging markets and is thus only purchased by a minority, i.e., the most affluent sections of the population. Nevertheless, wet food enjoyed strong growth in some emerging markets, most notably Mexico.

**Mars/Nestlé ‘duopoly’**

The global market for pet food and pet care products remains concentrated in the hands of two main manufacturers—Mars and Nestlé. Recent takeovers, i.e., that of Ralston Purina by Nestlé during 2001 and that of Royal Canin by Mars one year later, further added to their already prevalent ‘duopoly’. Nevertheless, growth surge in premium and superpremium cat and dog food saw other manufacturers gain share in 2001, most notably Procter & Gamble, which benefited from an expansion of Iams into supermarkets during 2000 and 2001. Mars and Nestlé are primarily present within the mid-price segment, but both companies focused strongly on establishing themselves also within the premium segment, partly through product reformulations, and partly through the takeovers.

**Global outlook**

The ‘recession proof’ pet products market is set to continue to grow strongly in developed and mature markets. Over the 2002-2007 period, value sales are forecast to grow to US$51 billion, as a result of increasing pet ownership levels, rising consumption levels of industrially prepared food in developing markets and consumers moving to premium products in developed markets (Table 2, Figure 3).

**Table 2. Pet products: global future outlook.**

- Growing market (+16% or US$8 billion)
- New products in North America and Western Europe
- Functional pet food
- China to drive growth in Asia Pacific
- Educational programs
- Still a large potential to explore for further growth

**Figure 3.** Sales of pet products in the world market: forecast to 2008 (Combelles, 2003).
References

Using nutritional genomics to study canine obesity and diabetes

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Incidence of obesity and diabetes

According to the 2000 National Health and Nutrition Examination Survey (NHANES), approximately 64% of American adults (>20 years of age) are either overweight (>25 body mass index (BMI)) or obese (>30 BMI) (CDC, 2003). Although a genetic component exists, behavioral patterns and food availability are the major factors in the recent increase in obese populations. Americans are eating more than previous generations due to the availability of a wide variety of good-tasting, inexpensive, energy-dense foods. Lower amounts of exercise at work, school, and home are also to blame. Obesity has been equated with aging 20 years, being more strongly linked to chronic diseases than living in poverty, smoking, or drinking (Sturm, 2002). Obesity is a major risk factor for several life-threatening diseases such as heart attack, stroke, non-insulin dependent diabetes, and certain cancers (e.g., colon, breast), leading to approximately 300,000 obesity-related deaths each year in the US (Marx, 2003).

Closely associated with obesity is diabetes, which continues to increase in the United States and other developed nations. Approximately 6% of Americans have diabetes and an equal number of people are thought to be in a ‘pre-diabetic’ state (Marx, 2002). Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia and disturbances in carbohydrate, fat, and protein metabolism associated with absolute or relative deficiencies in insulin production by pancreatic β-cells and/or insulin action at target tissues (Bennett, 1994). Several pathologies are responsible for the development of diabetes, which can take several forms. The majority of human diabetes cases fall into two broad etiopathogenetic categories. The first (type 1) is due to an absolute deficiency of insulin secretion, whereas the second (type 2) is due to a combination of resistance to insulin action and an inadequate compensatory insulin secretory response (ADA, 2002). Type 2 diabetes, also referred to as non-insulin dependent diabetes, is by far the most common form, accounting for approximately 90% of cases (Warram et al., 1994). Diabetes increases mortality and morbidity rates, as it is associated with several complications including heart disease and stroke, blindness, amputations, renal disease, and damage to the nervous system.

The parallels that exist between dogs and humans as regards recent lifestyle changes, increased lifespan, and increased incidence of obesity and associated diseases are remarkable. Obesity is likely the most common disease found in companion animals today. Up to 40% of dogs presented to veterinarians in the US are now overweight, which is significantly higher than just a few decades ago (Sunvold and Bouchard, 1998). Because the anomalies associated with canine obesity and diabetes are intertwined, it is not surprising that the incidence of diabetes also has increased by 3-fold in this same period (Guptill et al., 1999). Immune-mediated diabetes (type 1) is the most common form found in dogs (Hoenig and Dawe, 1992). Although the type 2 (non-insulin dependent) form accounts for only 1 in 5 canine diabetic cases (Feldman and Nelson, 1996), the etiology and gross clinical signs are similar to those of humans (Hoenig, 1995). This similarity will prove to be beneficial for both species, as dogs are important biomedical models for several human diseases. Conversely, clinical and nutritional information collected from human experiments may be applied to canines to improve nutritional and health status.
The dog has been crucial in understanding glucose metabolism, pancreatic function, and diabetes, as it was the first animal to become diabetic experimentally and was the animal used to determine pancreatic function (Mering and Minkowski, 1889). The cause of obesity in dogs is similar to that in humans; inadequate daily exercise and excessive intake of high quality foods (Mason, 1970). Many of the negative health outcomes of obesity observed in humans also are present in the dog. Weight gain is associated with increases in blood pressure, heart rate, plasma volume, cardiac output, and fasting insulin concentration (Rocchini et al., 1987). Many of the complications associated with diabetes in humans, including hypertension (Struble et al., 1998), hypercholesterolemia (Barrie et al., 1993), atherosclerosis (Sottiaux, 1999), and retinopathy (Wyman et al., 1988), also are present in canines. In fact, the dog is a popular model for ocular manifestations because diabetes causes cataracts and is the leading cause of blindness in dogs (Wyman et al., 1988). Finally, clinical signs of diabetes are similar to those of humans, with polydipsia and polyuria being the most common signs in newly diagnosed diabetic dogs (Plotnick and Greco, 1995).

Factors influencing incidence of obesity and diabetes

The pathogenesis of obesity and type 2 diabetes is a complex process that occurs over an extended period of time in both humans and dogs. Age, genetic profile, and dietary/lifestyle habits are highly associated with these metabolic disease states. In humans, diabetes affects only 0.2% of people under the age of 20, 8.6% of those between 20 and 65, and 20.1% of people over 65 years old (www.cdc.gov/diabetes/pubs/estimates.htm). Dogs may develop diabetes at almost any age, but it is most common from 7 to 9 years of age (Nelson, 1995). Mattheeuws et al. (1984) reported that as dogs increased in age and became heavier in weight, regulation of plasma glucose and insulin worsened, which is similar in the human (Glass et al., 1981). Other reports also have suggested poor regulation of glucose metabolism in geriatric dogs (Sheffy et al., 1985; Mosier, 1989).

Nutrition also plays a major role in the development of diabetes. Over-consumption of food by normal-weight subjects, resulting in weight gain and adiposity, has been shown to induce hyperinsulinemia in animal models and humans (Simms et al., 1973). This relationship is corroborated by the fact that over 80% of human diabetics are also obese. Knowler et al. (1990) reported that individuals having a BMI >35 were 100-300% more likely to develop diabetes compared with those having a BMI <25. The presence of obesity is not always a positive predictor of diabetes, however, as only 10% of obese people are diabetic (Beck-Nielsen and Hother-Nielsen, 1996). Diabetes resulting from over-nutrition is also common in dogs, as most dogs diagnosed with diabetes are overweight. In addition to total caloric intake, the type of food consumed also may affect the development of diabetes. Several animal experiments have demonstrated that glucose metabolism and homeostasis are influenced by high fat consumption. Several researchers have measured the glycemic and insulinemic responses of pet foods having different carbohydrate (e.g., starch, soluble and insoluble dietary fiber), fat, and protein contents or prepared using different processing methods (e.g., canned, soft moist, dry) (Holste et al., 1989; Nguyen et al., 1998). These experiments usually were performed to identify diets useful for managing dogs suffering from obesity and diabetes. However, little has been done to identify ingredients that may contribute to the development of canine diabetes.

Finally, genetics plays an important role in the development of diabetes, as its incidence in racial groups differs substantially. The incidence of type 2 diabetes in African Americans, Hispanics, and whites living in the US is approximately 13, 10.2, and 6.5%, respectively (Marx, 2002). In comparison, approximately 50% of the Pima Indians of Arizona have the disease. Numerous genetic loci affecting diabetes susceptibility, unrelated to racial categorization, also likely exist. Genotype (genetic makeup) also plays an important role in canine metabolic disease states such as obesity and diabetes. Breeds such as Labrador retrievers, Cairn terriers, cocker spaniels, long-haired dachshunds, Shetland sheepdogs, basset hounds, Cavalier King Charles spaniels, and beagles, have a greater prevalence of obesity than other breeds (Mason, 1970; Edney and Smith, 1986). In another experiment, Samoyeds, miniature schnauzers, miniature poodles, pugs, and toy poodles were found to be at high risk for developing diabetes, while German shepherds, golden retrievers, and American pit bull terriers were at low risk (Hess et al., 2000). Specific genetic mutations resulting in diabetes at a young age also have been identified in dogs. Inherited insulin-dependent diabetes has been identified in keeshond and Samoyed.
breeds (Kramer et al., 1980; Kimmel et al., 2002). A mutation in the glucose-6-phosphatase gene, a key regulatory enzyme in glucose homeostasis, also has been identified in dogs (Feng et al., 1997; Kishnani et al., 1997). As more genes and genetic polymorphisms (genetic variants) are identified in the canine genome, other populations prone to obesity and diabetes may be identified.

**Metabolic diseases are highly complex and difficult to study**

Despite substantial investments of time and money by many laboratories, the identification of genes contributing to type 2 diabetes has been difficult (Elbein, 1997; Neel, 1999). Type 2 diabetes frequently goes undiagnosed for several years because hyperglycemia develops slowly and is not severe enough in early stages for the patient to notice the classical signs associated with the disease (e.g., polyuria, polydipsia, polyphagia, weight loss) (ADA, 2002). Type 2 diabetes often is not diagnosed until overt diabetes, an irreversible part of the disease cycle, is present. This may be especially true for companion animals because owners may not notice clinical signs of disease until it has progressed to an irreversible stage. Therefore, screening tools are needed to identify the progression of diabetes that is undetected by serological indices (e.g., glucose and insulin concentrations). Although fasting plasma glucose and other serological indices may be helpful in that they are able to detect glucose intolerance and early stages of diabetes, they are not able to predict its development. Screening tools able to predict the disease are needed so nutritional and exercise strategies may be implemented to prevent or prolong its development. Gene expression profiling may be a method to detect early stages of diseases such as diabetes before gross clinical signs are noticeable.

Because previous efforts to identify genetic loci responsible for the complex pathogenesis of obesity and type 2 diabetes have had little success, a better understanding of metabolic pathways in healthy individuals may be needed before confronting such complex metabolic diseases. Nutritionists are beginning to use the powerful molecular biological techniques available, but the nutritional genomics field is still in its infancy. Metabolic pathways such as the glycolytic pathway, which was completely elucidated in 1940, have been studied to a great extent (Stryer, 1995). Substrate and enzyme structures, regulatory sites, and regulatory elements such as insulin and glucagon have been described. However, completely understanding the regulation and interaction of metabolic pathways is a complex problem that will not be easily solved. Energy balance and whole body metabolism is a complex process involving numerous genes and proteins that interact to determine metabolic status. The fact that most mammalian genomes are estimated to contain only ~30,000 to 40,000 genes suggests that in addition to the overall number of genes present in a genome, other factors such as temporal and spatial gene expression patterns, alternative splicing, post-translational modification, and protein-protein interactions, greatly influence phenotype (physical characteristics). Recent advances in molecular biology, including high-throughput tools used for gene and protein functional analyses, will be instrumental in describing and characterizing normal and diseased metabolic states.

**Importance of canine genome sequencing**

Because genomic data are often needed to implement the use of the emerging molecular biological techniques, canine genome sequencing is crucial. Because of their similarities to human physiology and genome structure, the dog is a powerful biomedical model. Canine models of human diseases are important in identifying disease-causing genes, analyzing gene and protein function, and developing treatment strategies. As approximately 50% of canine genetic diseases have a human counterpart, researchers have demonstrated the dog’s utility as a biomedical model of numerous human diseases including obesity and diabetes (O’Brien et al., 2002). The importance of the dog as a biomedical model is indicated by its rank in the National Human Genome Research Initiative (NHGRI) genome sequencing scheme (http://genome.gov). The dog is the first nonrodent mammalian animal model to be sequenced and is expected to be completed (6.5 X coverage) by June 2004 (www.genome.wi.mit.edu/media/2003/pr_03_tasha.html). In addition to the canine sequencing funded and coordinated by NIH, a privately-funded project recently sequenced ~70-80% of the canine genome (Kirkness et al., 2003). In addition to the continual supply of sequence data added to the public databases, researchers have access to canine genetic maps. Breen et al. (2001) published the first fully integrated, comprehensive map of the
canine genome. This 1800-marker map (containing 320 genes and 1078 microsatellites) covered >90% of the canine genome. Shortly thereafter, Guyon et al. (2003) published another map of the canine genome that contained 3270 markers. These established genetic maps are crucial in ordering genes in the canine genome as the sequence information continues to be added to public databases. Comparative mapping with human and mouse genome sequences, which have already been completed, will increase the speed at which this process occurs in dogs.

Although sequencing the dog genome was initiated because of its importance as a biomedical model for humans, dogs and their owners also will greatly benefit from this information. The most immediate impact of genome sequencing may be the detection and elimination of canine genetic diseases by identifying genes responsible for them. At present, approximately 450 canine genetic diseases have been identified (http://www.angis.org.au/Databases/BIRX/omnia). Test results may be used to eliminate carriers from the breeding population in order to decrease or eliminate incidence of disease. Due to high sequence homology among dogs, humans, and mice, comparative mapping will be helpful in correctly placing the genes in the canine genome. Once sequence information is known, genetic polymorphisms may be identified. Further testing will be required to identify which of these polymorphisms are important in health and disease. Some common genomic terms are presented in Table 1.

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### Table 1. Common genomic terms.

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Functional genomics</td>
<td>the study of the function of every gene encoded in a genome.</td>
</tr>
<tr>
<td>Genome</td>
<td>the totality of all DNA in an organism.</td>
</tr>
<tr>
<td>Genomics</td>
<td>the study of genomes, including genome mapping and sequencing.</td>
</tr>
<tr>
<td>Genotype</td>
<td>the genetic makeup of an organism.</td>
</tr>
<tr>
<td>Nutritional genomics</td>
<td>the study of nutritional effects on gene expression.</td>
</tr>
<tr>
<td>Phenotype</td>
<td>the physical characteristics of an organism.</td>
</tr>
<tr>
<td>Polymorphism</td>
<td>a variation in DNA sequence.</td>
</tr>
<tr>
<td>Proteomics</td>
<td>the study of the full complement of proteins found in an organism.</td>
</tr>
<tr>
<td>Transcription</td>
<td>the creation of a messenger RNA strand from a DNA strand.</td>
</tr>
<tr>
<td>Translation</td>
<td>the creation of a protein from a messenger RNA strand.</td>
</tr>
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### Utility of functional and nutritional genomics

Although the progress made in the past decade as regards genome sequencing has been astounding, the vast amount of information it provides comes without interpretation. Genome structure is important, but the function, regulation, and interaction of genes and gene products (proteins) have the major influence on phenotype. Therefore, assessing gene function by analyzing RNA and protein expression, localizing proteins, and determining the significance of protein-protein interactions are of major importance (International Human Genome Sequencing Consortium, 2001). As the field of functional genomics and proteomics (study of protein profiles) matures, our understanding of gene and protein function, cellular function, and physiology will be greatly enhanced. This newfound knowledge will allow scientists to fully utilize genome sequence data and lead to an improved understanding of the biological systems of the body and subsequently develop effective prevention and (or) treatment therapies for disease. This area of research also will be very important for the field of nutritional sciences. If applied correctly, nutritional genomics will enhance our understanding of metabolic pathways and aid in maximizing dog nutritional and health status.

Dietary constituents can up-regulate or down-regulate gene expression directly, as is the case for certain vitamins and minerals, or by indirect means (e.g., dietary fiber) through hormonal signaling, mechanical stimuli, or metabolites produced from gut microflora (Cousins, 1999). Changes in gene expression have been used to study a broad range of topics, including caloric restriction (Lee et al., 1999), vitamin (Nur et al., 2002) and mineral deficiencies (Blanchard et al., 2001), glucose metabolism (Uyeda et al., 2002), and diseases affecting nutritional status (Gannon and Nuttall, 1997). However, the effects of nutrition on gene expression in companion animals have not yet been tested. Several areas of companion animal nutrition are not well studied, but experimental work may be enhanced by using nutritional genomic techniques, including the determination of minimal, optimal, and toxic concentrations of all nutrients, efficacy and toxicity testing of novel ingredients, effects of nutrition on development, prevention, and treatment of complex diseases, and genetic polymorphisms important for nutritional metabolism and requirements and susceptibility to diseases.

Genomic technology may be used to identify genetic and dietary factors responsible for the development of a complex disease and aid in the development of prevention and treatment strategies and therapeutics. Continued advances in
biotechnology have provided scientists with powerful research tools that will generate more informative and less invasive research. Many of these new tools have been in the area of functional genomics, the study of assessing gene function. One of these techniques, microarray technology, has become very popular because it enables researchers to measure the expression of hundreds to thousands of genes simultaneously (Pease et al., 1994; Schena et al., 1995). This snapshot of gene expression provides a global perspective of the cellular or tissue metabolic state, rather than measuring only a few genes at a time, which limited classical techniques. By using this technology, gene expression profiles may be generated and correlated with metabolic indices from blood or tissue samples to identify relationships important to understanding whole body metabolism.

To be less invasive and provide more accurate analysis, several microgenomic techniques also have been developed in the past decade. Laser capture microdissection (LCM) enables researchers to isolate and study pure cellular populations from a heterogeneous tissue. In combination with RNA isolation and amplification procedures, LCM has become a very important tool for researchers and clinicians with small sample sizes (Simone et al., 1998). Because LCM allows researchers to perform cell-specific genomic analysis on small samples such as biopsies, this technique has become very popular in cancer and gastrointestinal research. By using this technology, gene expression profiles may be generated and correlated with metabolic indices from blood or tissue samples to identify relationships important to understanding whole body metabolism.

Rather than attempting to tackle complex metabolic diseases such as obesity and diabetes immediately, comparison of gene expression profiles from simple differences in dietary status or composition (e.g., fasted vs fed, animal vs plant-based ingredients) may be the place to begin. Once relationships of normal metabolic status are established, gene expression profiles or ‘signatures’ of disease may be identified in future experiments. These signatures will reveal abnormalities in metabolism that may be important for understanding disease development, developing biomarkers of disease, and choosing effective therapies. Due to the lack of knowledge regarding the canine genome and the expense involved with microarray technology in the past, canine microarrays have been available only on a proprietary basis at an extremely high cost. However, increased knowledge in both key areas (canine genome, microarray technology) in the past decade is enabling the production of canine microarrays using gene-specific probes. Because options are currently limited, canine microarrays are still very expensive ($400 to $500/slide). However, as competition in the marketplace increases, these costs will likely decrease. By generating gene expression profiles of canine blood and liver samples already known to have metabolic differences (but in healthy animals), relationships between these datasets may be established. Along with understanding gene function and the effects of gene-gene interactions, these relationships will lay the foundation for future experiments studying complex diseases such as obesity and type 2 diabetes.

**Illinois canine nutritional genomics experiment**

Our laboratory recently conducted a 12-month experiment evaluating the effects of diet on gene expression in healthy elderly (11 years old at baseline) and weanling (8 weeks old at baseline) dogs. Due to the lack of knowledge in this area, this initial experiment was designed to evaluate major differences in age and diet on canine gene expression profiles. In this experiment, an animal product-based diet was compared with a plant product-based diet. Blood and liver biopsy samples were collected over the course of the experiment and tissue samples were harvested at its end for isolation of RNA to be used for microarray analysis. In addition to RNA isolation, blood samples were used for determining serum metabolite concentrations and complete blood count. Fecal and colonic digesta samples were used to measure nutrient digestibility and fermentative end-product concentrations. Tissue samples of various regions of the gut also were collected for the measurement of gut morphology and for RNA isolation using LCM, which will generate intestinal villus cell- and crypt cell-specific gene expression profiles. Our primary goal was achieved, as several metabolic differences due to age and diet were identified. Nutrient digestibility, hematologic, and serum chemistry data were recently submitted for publication (Swanson et al., 2004).

We are currently in the process of generating gene expression profiles that may be correlated with the metabolic data already collected. Significant
Using nutritional genomics to study canine obesity and diabetes

Correlations may identify mechanisms responsible for changes observed in biological systems (e.g., metabolic pathways, immune function) due to age and diet. These results also will be used to design future experiments in our laboratory evaluating the effects of nutrition on obesity and diabetes. We plan to identify biomarkers of disease and test dietary regimens that may prevent disease or improve nutritional and health status.

Conclusions

Now that pets are living longer lives due to improved nutrition and veterinary care, the incidence of complex metabolic diseases such as obesity and diabetes has increased. These complex diseases are difficult to study experimentally because of the time they take to develop and the various environmental and genetic factors involved. However, emerging genomic technologies are enabling scientists to perform more definitive and less invasive research that may enhance our understanding of normal and diseased metabolic states. Nutritional genomics may enable researchers to identify biomarkers of these diseases so they may be detected in early stages and treated appropriately. The current canine genome sequencing initiative will be crucial to improving canine health, as it will allow researchers to locate and determine the function of genes and identify genetic polymorphisms that influence metabolism, immune status, and other biological systems. These technologies will not only identify populations prone to disease, but will play an important role in developing strategies for prevention and (or) treatment.

References


K.S. Swanson


A peek into the new NRC for dogs and cats

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Introduction: new directions for the NRC book for companion animals

Three years ago, I spoke to this audience with great anticipation of the release of the National Research Council’s *Nutrient Requirements for Dogs and Cats*. I identified areas of nutrition research where gaps still existed in our knowledge and tried to predict some of the new information that might appear in the new NRC publication. While the release of the final document has been delayed until mid-2004, a pre-publication document was made available in November of 2003 (NRC, 2003). My excitement diminished after looking at the pre-publication. There is a tremendous amount of good work in the pre-publication; but there were parts that gave me, as an end user of the document, serious concern. Let me stress to anyone attempting to use the pre-publication document, that it is NOT the final version. The NRC staff has been resolute that the final document will look exactly like the pre-publication document with the exception of editorial changes. I cannot predict what will be deemed an editorial change and what will not. My comments are based on the pre-publication version with caveats. I have quoted the pre-publication document (as NRC, 2003) and these cited sections might be changed in the final version.

It has been nearly 19 years since the 1985 NRC Nutrient Requirements for Dogs was released. That edition contained 79 pages. The 1986 NRC Nutrient Requirements for Cats contained 78 pages. The 2003 pre-publication version of the new combined dog and cat edition contained 447 pages and had no index (NRC, 2003). The difference between the editions shows the immense amount of work done by the contributors to the document. The text was greatly expanded. The review of literature was extensive and the references are given. There are entirely new sections. The discussions include not only essential nutrients, but also non-essential nutrients. There are synergies in the combination of the two species allowing for the combination of basic information. However that same combination is often confusing in its organization, leaving the reader struggling to be sure which species is under discussion.

Each edition of NRC publications has a new and different expert committee chosen to write the publication. Each committee gets a slightly different charge for its job. The 2003 committee was told to use only peer-reviewed documents. The committee writing the 1986 NRC for cats was able to use ‘published research and practical experience’ (NRC, 1986). As quoted from the 2003 pre-publication overview, “This edition differs from the others because it contains the latest data on requirements, based on the utilization of nutrients in ingredients commonly produced and commercially available in dog and cat food rather than only on purified diets.” The nutrient requirement tables in the 1985 dog book and the 1986 cat book were based on purified diets and at least in the cat publication, “A margin of safety was intentionally not incorporated...” (NRC, 1985; 1986). Thus, the nutrient requirement tables in the two earlier editions could not be put into practical use without adjustments and could not be used directly for regulatory purposes. This is one reason that the AAFCO Dog and Cat Food Nutrient Profiles were developed (AAFCO, 2004). In the text of the new edition, comments noted that for some values adjustments have been included to account for bioavailability, but not for all values. So, the 2003...
publication appears to be related to practical diets but not in a consistent manner. It is important to remember that the nutrient values are recommendations for healthy animals and normal function, not for animals in disease states. The 2003 committee was made aware that the publication would be promoted for a wide variety of audiences: professionals in the industry, academia, students, government officials and pet owners. Thus, “...the committee chose to err on the side of caution and include adequate detail from the literature cited to provide a clear roadmap for how the recommendations were derived” (NRC, 2003). The publication is a serious scientific document addressing the complexity of nutritional science for dogs and cats. While there are knowledgeable individuals in the public audience, the publication is not casual, accessible reading. There is a vast amount in this new edition to digest. The initial review of the pre-publication version by industry professionals found errors and raised serious questions about parts of the document. As with previous editions, the tables in this book must not be used in isolation from the text. The values in the tables are based on certain assumptions about energy content and do not translate directly to most products in the marketplace. This is especially important for government regulators, both in the US and in other countries, to know and understand. The Pet Food Institute has already received numerous inquiries from other countries wishing to use the publication for regulatory purposes. Regulators in the US are planning a review of the practical, usable AAFCO Dog and Cat Food Nutrient Profiles, once the final NRC document is available (AAFCO, 2004). That does not necessarily mean that the AAFCO Nutrient Profiles will change. It means that the latest information will be considered and may be incorporated. Other countries, in the past, have not used the AAFCO Profiles, but continued to rely on the NRC minimum requirement values to judge the nutritional adequacy of pet products. The values in the tables in this new edition of the NRC document should not be used directly for regulatory purposes. This is a scientific document, not a regulatory document. I cannot state that strongly enough. Finally, some of you will have seen the newspaper headlines the morning after the press conference on the pub-publication release. “Pudgy Pets are Packing on Pounds,” cried the Wall Street Journal (Foley, 2003). “Chew on This: Pets are Pudgy, Too” exclaimed the Los Angeles Times (Mestel, 2003). Yes, the publication does include a handful of paragraphs on obesity in dogs and cats in the more-than-400 pages, but the vast majority of the document is NOT about obesity. As you will see below, it is about much more.

New topics included

Dogs and cats are complex organisms with many factors influencing their nutrient needs. This new edition tries to tell the entire story. The animal must eat the food, digest the food, absorb the components of the food and the body has to metabolize those components. In addition, nutrient needs are controlled by physical activity and various environmental factors. Four entirely new topics have been included: comparative digestive physiology, feeding behavior, special considerations for laboratory animals, and physical activity and climate. The new topics emphasize the broader view of this publication compared to past ones. No longer are the minimum requirements for the essential nutrients the entire purpose of the document. All of these factors are covered in the new edition, which should be considered a strength of the publication.

Changes to the tables of requirements

The traditional table of requirements, which in the past listed minimum requirements for growth and maintenance, has become multiple tables expanded to include reproduction. Each table now has a complicated four-category system of values, depending on the information available: minimal requirement, adequate intake, recommended allowance and safe upper limit. The requirements have new assumptions for specific body weights, and in lactation for specific numbers of kittens/puppies. Footnotes are given for adjustment of the values when body weights or numbers of offspring change. There are new units appearing in the tables as well. Where the previous edition utilized percentages or parts per million on a dry matter basis plus amounts per 1000 kcal ME; the new edition has four types of units. The requirements are represented as: amount per kg dry matter, amount per 1000 kcal ME, amount per kg body weight and amount per kg metabolic body weight (BW^{0.75}). These extra units were included to assist veterinarians in customizing dietary support for individual patients. In short, the tables look significantly different from those of past editions.
Essential nutrients

In my previous paper, I identified the following gaps that I thought might be covered in the new edition: taurine for cats (would such be listed for dogs?); linoleic acid for dogs; levels of calcium and phosphorus for large breed dogs; copper requirements for reproducing cats; and finally, vitamin K for cats. I suspected that some of the other gaps in nutrient requirements would remain gaps. These included manganese for dogs, iodine and selenium for cats, the effect of interactions of minerals on availability, and vitamin requirements for reproduction.

Taurine was included as an essential nutrient for cats with values given for minimal requirements. Taurine has been extensively researched in the years since the last NRC edition. This research is well recognized in the publication. Taurine needs in dogs are discussed in the text, but a minimal requirement is not included in the table. The committee did not find peer-reviewed, dose-response experiments that supported the establishment of a minimal requirement. They did comment on the practice of inclusion of taurine in lower protein diets for dogs, which allows the maintenance of taurine pools.

Calcium and phosphorus levels in puppies of different breeds are discussed, which is good news. But the bad news is that the user of the tables must carefully read the text in order to set values in diets. For example, a minimum calcium requirement for puppies is set in the table, with a footnote which states “For calcium and phosphorus, the need may decrease by up to 20% per unit energy between 60 and 100% of mature body weight. Values are for giant breeds and may be different in others (see text)” (NRC, 2003). This is an example of the complications inherent in setting nutrient requirements. The table of requirement values for growth of puppies is set using a model 3-month old puppy weighing 5.5 kg, consuming 1000 kcal ME per day, but the footnote says the calcium and phosphorus values are set for giant breed puppies. The last 3-month old St. Bernard puppy I saw weighed closer to 18 kg than 5.5 kg. A reading of the text shows “...a minimum requirement of 2.0 g Ca per 1000 kcal ME (8.0 g Ca per kg, 4000 kcal ME per kg) may be set as the minimum requirement of dietary Ca that will support normal growth in puppies of both large and small breeds.” The table value for minimal requirement is indeed 2.0 g Ca per 1000 kcal ME. The text additionally says that the recommended allowance of 3.0 g Ca per 1000 kcal ME (the value in the table) “should be sufficient for all breeds.” Why then the footnote? Perhaps the second part of the footnote refers to only one column of the table, which then would be more consistent with the text.

While there is a long discussion of fatty acids in the text (about which more will be discussed later), minimal requirements are not set for linoleic acid in either species, nor are minimal requirements given for arachidonic acid for cats. Adequate intakes are given for linoleic and arachidonic acids for both species. As I said three years ago, this gap in research on known essential fatty acids seemed so basic, and yet the needed research just was not done to allow the establishment of minimal requirements.

Despite some relevant research, a minimal requirement for copper is not set for cats during reproduction citing “…the absence of specific experimental data…” (NRC, 2003). The available data were reviewed, deemed not specific enough and only an adequate intake is listed in the table. Many of the trace minerals are in the same situation, adequate levels are set but not minimal requirements. This is the case for both dogs and cats. The greatest number of minimum requirements for trace minerals is set for growth in both species. Clearly, trace mineral research is still an open area.

Several vitamin requirements remain open for both species. I had speculated that a vitamin K requirement might be set for cats, but while there are some data, the committee’s reports were not enough to set a minimal requirement value. Only one minimal vitamin requirement is set for dogs, which is for riboflavin in adult maintenance.

Although research had been done for various essential nutrients, the experiments did not fulfill the committee’s criteria needed to set the minimal requirements.

Non-essential nutrients

One of the two biggest categories of non-essential nutrients identified for consideration three years ago was omega-3 fatty acids (referred to in the text as n-3 fatty acids). The 2003 pre-publication did recognize the importance of this area. The text discusses not only the details of the n-6 fatty acids: linoleic and arachidonic acids, but also includes the n-3 fatty acids: alpha-linolenic (ALA), eicosapentaenoic (EPA), and docosahexaenoic (DHA). The text states “Both n-6 and n-3 fatty acids are essential for dogs and cats.” However, it also states “A specific requirement for
long-chain n-3 PUFAs (EPA and DHA) in adult dogs has not been identified to date.” Under some circumstances certain fatty acids were noted as conditionally essential. All of the above fatty acids are listed in the tables for both species and adequate intake values, (not minimal requirements) are set for all. Does inclusion in the tables recognize all these fatty acids as essential or not?

There is a listing for the combination of EPA and DHA in each of the tables. The tables for adult dogs, reproducing dogs and adult cats include a footnote regarding a general recommendation about the appropriate mixture of EPA and DHA for diets, at 30% of each. What then should the other 40% of n-3 fatty acids be? How was this ratio established? The following statement is in the text section on adult maintenance of dogs “A general recommendation is that EPA and DHA should contribute approximately one-third each of the total dietary n-3 LCPUFAs.” No reference is given as to the source of this general recommendation and yet the charge to the committee was to only consider peer-reviewed publications.

The second big category identified for consideration three years ago was carbohydrates and fiber. The 2003 committee did a great deal of good work in this area. Carbohydrates as a nutrient category were covered in less than four pages total in both previous editions. That has now been expanded to 35 pages with a complete discussion of all groups in the carbohydrate family. The category was divided into four groups: absorbable, digestible, fermentable and non-fermentable. The text is quite detailed in its discussion of the utility, nutritive value, effects on growth and reproduction and physiological effects of each group of carbohydrates. It is interesting to note that while many important nutritional aspects of carbohydrates are discussed, there are no carbohydrates included in the tables; thus leaving the impression that while they play a definite role in dog and cat nutrition, they are still not considered essential nutrients. From the US regulatory perspective, the only recognition of carbohydrates in regulation is as crude fiber, a term not used in the text of the carbohydrates chapter. So carbohydrates are important in nutrition, but not classically essential, and not a simple nutrient class for regulatory attention.

Energy

The topic of energy received vast research attention between editions of the publication. As in the carbohydrates section, the 2003 committee made a great effort in the energy chapter. The four pages in the 1985 NRC for dogs and the three pages in the 1986 NRC for cats have become 26 pages in the 2003 edition. (NRC, 1985; 1986; 2003) The Holy Grail has been one energy requirement calculation equation for each species, which is clearly not appropriate based on the research. This is reflected in the tables, where energy requirement calculation equations for each species at each life stage are provided. These equations are much more sophisticated than those given in earlier editions. This approach is entirely consistent with the goal of providing end users the ability to individualize energy delivery to animals. However, for regulatory purposes in labeling products with feeding directions, this level of complexity presents problems. The AAFCO Model Pet Food Regulations require that feeding directions be given on all pet foods labeled as complete and balanced for any and/or all life stages (AAFCO, 2004). These directions must be consistent with the intended use of the product. This means that if a product is labeled for all life stages, then feeding directions must be given for all life stages. In the 2003 NRC, there is one equation for weaned puppies and one equation for adult dogs but six multiplication factors determined by the dog’s activity level and breed. For late gestation, there is a two-part equation, while for lactation, there is another two-part equation that depends on the number of puppies and week of lactation. Those equations are all scientifically sound, but it will be difficult for a non-expert regulator to judge the appropriateness of the feeding directions on a label. In addition, how extensive do label feeding directions need to be? This will be for AAFCO Pet Food Committee and industry to work together to balance accurate science with usability for the consumer. This is another reason why the nutrient requirement tables in the 2003 publication should not be put directly in to use as regulation.

Safe Upper Limit (SUL)

This is the first appearance of SULs in the NRC for dogs and cats. In fact, SULs have not appeared in previous NRC publications such as those for swine, dairy cattle or beef cattle (NRC, 1998; 2000; 2001). Those documents used the carefully defined words “maximum tolerable concentration” and did not include values in the requirement tables. There were too many conditions related to every value that had
to be considered when applying it to a diet. The 2003 NRC dog and cat publication defines the SUL as “the maximal concentration or amount of a nutrient that has not been associated with adverse effects...” The inclusion of SULs in the tables is of great concern to industry professionals. The concept of upper limits is not foreign to the industry. The AAFCO Dog and Cat Food Nutrient Profiles include a few maximum levels for nutrients where valid concerns about toxic levels exist (AAFCO, 2004). Industry review of the pre-publication raised serious doubts about the usage of SULs as they appear in the 2003 NRC.

The logical interpretation of ‘safe upper limit’ by government officials and the public is that any product containing nutrients at levels higher than those given in the publication will harm animals. But that is not the way the values were developed. This is a major disconnect. Perhaps at least the name should be changed to better reflect the concept as used by the NRC. The origin of the values throughout the publication appears inconsistent, which indicates that the meaning of the SUL is inconsistent. For example, three of the applications of the SUL concept are:

- absolute maximum where toxicity exists;
- upper level tested in research;
- an upper level tested in research with a safety factor added.

In some cases, the SUL represents the highest level tested in the research, but often no higher level was tested or shown to result in adverse effects. Thus, the highest level tested was set as the SUL, without knowledge as to whether or not a higher level would result in adverse effects. This seems misleading. Some of the SUL values in the tables show a ‘greater than’ symbol in front of the value. Examples are zinc for adult cats, amino acids for kittens, lysine for puppies, sodium and iodine in adult dogs, etc. This appears to mean that the SUL is some unknown value above the value given. I do not see that including such a value in the table is reasonable. No limit is defined. Perhaps a footnote without a value noting that there is concern about potentially toxic levels for that nutrient would be appropriate.

Another concern with SUL is that some values were set based on a general rule of thumb, which is not equivalent to the “maximal concentration...not associated with adverse effects.” For example, the SUL for thiamin in both species was set based on a suggestion by NRC that the presumed safe upper limit would be 1000 times the dietary requirement (NRC, 1987). The text states “There are no reports of toxicity resulting from excessive oral intakes of thiamin, but intravenous thiamin in high doses can cause neuromuscular and ganglionic blockade” (NRC, 2003). If the SUL is for parenteral nutrition and not practical commercially available pet foods (the stated intent of the publication), then why is the value included or why is it not at least footnoted to acknowledge that fact?

Also of great concern is the inclusion of an SUL for total fat in each of the tables. This indicates that dietary levels above the stated value would be harmful to the animal. But the SUL for total fat was based only on the amount of fat that would result in a reasonable amount of space for protein at the given caloric content, not a direct safety concern for fat. In fact, the footnote for the SUL for total fat states “The upper limit of fat may be greater than values indicated because it is limited by protein concentration requirement in a diet designed to be 4 kcal ME per g DM. Any higher fat content would exceed this assumed caloric density.” (NRC, 2003). However for someone just looking at the fat level in the diet and looking at the table, a higher level of fat would be castigated as harmful. Perhaps this issue should be dealt with by inclusion of the footnote without a potentially misleading value. In the tables for dogs, there is a footnote for ALA in the SUL column. This footnote indicates a recommended ratio of LA to ALA and the ratio varies by life stage. In its discussion of SUL for LA and ALA, the text states “…so the ratio of LA to ALA is within the recommended range (see Table 5-I) “. There are no recommended ranges given in Table 5-I. Table 5-I is entitled “List of Abbreviations of Selected Fatty Acids…” The reference regarding the ratios may be in the text; it is certainly difficult to find.

One very troubling issue in the carbohydrates chapter was the last table, which set SULs for various ingredients for maintenance of adult animals. This is a clear departure from the rest of the publication. SULs in the rest of the document are set for nutrients, not ingredients. The inclusion of SULs for ingredients can easily result in the included ingredients being identified as ‘unsafe’. This is a disservice to very healthy and useful ingredients. The definition used here for SUL is “a level of dietary inclusion at which no adverse effects can be expected.” (NRC, 2003) However, of the 23 SULs set for dogs in the 2003 NRC publication, 13 of the levels had the following footnote, “Higher levels were not tested.” Therefore there really are no data to say whether these 13 values...
are or are not the SULs. This table also raises the potential for products currently in the marketplace, with histories of safe use, to be deemed ‘unsafe’, with no evidence that they are unsafe.

The concerns about SUL are not limited to the above, but they are some of the most visible issues. This concept and its use are of great concern. It is a serious, troubling possibility that government regulators or lay public could use SULs in inappropriate ways. SUL values, taken directly from the tables, cannot be utilized in a simple, across the board application to all products for the purpose of judging safety of the products.

**Conclusion**

I would like to thank the expert committee that put in many hours on a volunteer basis toward the completion of the publication. They were given a huge task. Much of the pre-publication document is worthwhile. However, there are areas of concern to at least one end user group, i.e., industry professionals. These areas of concern have been raised to the NRC staff and members of the industry have offered to work with the expert committee and NRC staff to help make this publication more sound and usable by the various target audiences. The NRC staff refused these offers.

I would also like to thank a group of very experienced nutrition professionals who dove in and reviewed the pre-publication document when it was released. These professionals came from all sectors of the industry. Their comments were valuable to me and I hope will be valuable in the future use of the final NRC publication.

We now await the final publication of the NRC Nutrient Requirements for Dogs and Cats. I think the peek at the pre-publication document gives us a very vital piece of information. There are still gaps in our knowledge of dog and cat nutrition. As noted in the overview, “Gaps in our knowledge of specific requirements are noted in the text and by absence of data in the requirement tables.” One of the goals for this NRC publication is for “…identifying new topics for research.” I think those gaps are evident. Nutrition research for dogs and cats remains an active arena. We all have many opportunities and a lot of work still to do.

**References**


The importance of antioxidant protection: demonstrating and branding benefits in pet food

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Animal nutrition in the last decade has seen great advances in the understanding and application of the correct balance of nutrients required in the diet and the quality of the raw materials used. In the companion animal sector, the focus on specific dietary requirements now means that pet owners have a huge choice of commercial diets of varying specificity and good nutritional quality available. Often the quality of pet food is given the same status as human food, as it is bought using the same purchasing decisions as the family’s weekly groceries. Scare regarding the use of chemicals and drugs in animal feed have heightened consumer worries regarding the safety and health benefits of their pet’s diet. This, coupled with the rise in interest in feeding for animal well-being, longevity and preventative medicine, makes the role of dietary antioxidants increasingly important as a maintainer of health status as well as a potential marketing opportunity.

Recently consumers have become more concerned with the quality and ingredients that are used in both human food and animal feed. The idea that natural or organically derived ingredients can help us and our pets remain healthy is particularly attractive, as these components are seen as being, by inference, safe to use, whilst having multi-functional but non-pharmaceutical benefits. Antioxidants fit very well as natural products strongly associated with health benefits, and are regarded by many as vital ingredients in animal diets. There is a growing body of research evidence into how antioxidants work and which dietary sources are the most potent, which lends technical weight to the justification for using such ingredients in commercial production.

Importance of counteracting oxidation

Oxidation is a necessary phenomenon of cell functions as oxygen is utilised in respiration, metabolism and energy production. However, oxygen is highly reactive and potentially toxic (Knight, 1998); and fundamental mechanisms such as respiration result in the production of oxidizing compounds called free radicals or ‘reactive oxygen species’ (ROS), the majority being in the chemical form O$_2^-$. These are highly unstable and reactive and facilitate various chain reactions that damage cell membranes and tissue function. Free radicals have been identified as causative agents for pulmonary hypertension syndrome, inflammation, cancerous tumour development and reproductive disorders. Essentially, free radicals reduce cell function and interfere with cell growth by damaging DNA, proteins, lipids (and hence membranes) and carbohydrates, which is why antioxidant deficiency is linked to a variety of disorders. Under normal, unstressed physiological conditions, between 3 and 5% of cellular oxygen is transformed into free radicals (Singal et al., 1998), an amount which increases dramatically under stress conditions. If the immune system is stimulated, free radicals are produced in large amounts during the proliferation of immune cells and can be used to damage and inactivate invading pathogens (Schwartz, 1996; Kettle and Winterburn, 1997; Surai, 2002), but will also cause further problems for the animal unless they are removed efficiently.

Preventing oxidation damage with antioxidants

Organisms have evolved specific antioxidant protective mechanisms that helped them to survive when oxygen concentration in the atmosphere was rising (Halliwell and Gutteridge, 1999). As a result there are thousands of naturally occurring compounds
possessing antioxidant properties to disable free radicals. These compounds work in various ways, as shown in Table 1.

### Table 1. Description of antioxidant compounds and precursors (adapted from Surai, 2002)

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Antioxidant function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E</td>
<td>Chain-breaking antioxidant, prevention of ROS proliferation, binding ROS</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Restriction of ROS propagation, Vitamin E recycling, ROS scavenging</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>Restriction of ROS propagation, immune function, mopping up excess ROS</td>
</tr>
<tr>
<td>Glutathione</td>
<td>Prevention of ROS formation by enzyme GSH-Px</td>
</tr>
<tr>
<td>Selenium</td>
<td>Prevention of ROS formation by enzymes: GSH-Px, thioredoxin reductase and other selenoenzymes, recycling vitamin E, DNA repair</td>
</tr>
<tr>
<td>Zinc</td>
<td>Prevention of free radical formation (superoxide dismutase enzyme)</td>
</tr>
<tr>
<td>Copper</td>
<td>Prevention of free radical formation (superoxide dismutase enzyme). Must be bound to protein to reduce oxidative potential</td>
</tr>
<tr>
<td>Manganese</td>
<td>Prevention of free radical formation (superoxide dismutase enzyme)</td>
</tr>
<tr>
<td>Iron</td>
<td>Prevention of free radical formation (catalase enzyme). Must be bound to protein to reduce oxidative potential</td>
</tr>
</tbody>
</table>

The main modes of antioxidant action can be split into three. The first is those compounds that prevent free radical formation in the first place. They do this by binding to co-factors required for formation of free radicals, such as iron. However, as is obvious from the immune example above, most reactions that generate free radicals are very important, so this is not always desirable. The second mode of action is the ability to neutralise excess free radicals once they have been formed, as seen in compounds such as vitamin E, and prevent the initiation of cascade reactions. The properties of such antioxidants can be measured via a standard test known as a Trolox Equivalent Antioxidant Capacity (TEAC) analysis, where their chelating strength is measured against vitamin E in a standard free radical solution. These compounds, however, can only bind a finite amount of free radicals, after which they become inactive. A constant dietary supply of antioxidant nutrients is therefore essential. This leads to the third mode of action, which is via the important antioxidant enzyme system in cells. These compounds work in concert with the chelating antioxidants by ‘recycling’ them – i.e. removing and neutralising the free radicals they have bound, reactivating the antioxidant. Two of the key enzymes involved in antioxidant are superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Certain minerals are integral components of these enzymes and are required in sufficient amounts from the diet to ensure synthesis in cell organelles to maximise the efficiency of antioxidation. For example, GSH-Px synthesis is highly dependent on selenium availability. There are two major forms of SOD in the cell: manganese SOD, which is located in mitochondria, and copper or zinc SOD which is found in the cytosol. This enzyme transforms the O$_2^-$ free radical to form hydrogen peroxide H$_2$O$_2$ rather than the more reactive radicals such as hydroxyl free radical OH*, which would occur otherwise.

\[
\text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \xrightarrow{\text{SOD}} \text{H}_2\text{O}_2 + \text{O}_2
\]

Hydrogen peroxide is more stable, but still toxic and must be removed from the cell by a further reaction, which is facilitated by either GSH-Px or catalase (CAT). Because GSH-Px is found in many cellular locations while CAT is located mainly in peroxisomes, the efficacy of H$_2$O$_2$ removal from the cell is greater with GSH-Px (Surai, 2002).

\[
\text{H}_2\text{O}_2 \xrightarrow{\text{GSH-Px (Se)}} \text{H}_2\text{O}
\]

As this first level of antioxidant defence in the cell is not sufficient to completely prevent free radical formation and lipid peroxidation, therefore a second level of antioxidant defence includes fat-soluble (vitamins A, E, carotenoids, ubiquinols) and water-soluble (e.g. ascorbic acid, glutathione, uric acid) antioxidants. These antioxidants are potent chain-breaking compounds, which prevent free radical chain formation and propagation. This mechanism is not straightforward. For example vitamin E reacts with a lipid peroxyl radical (LOO*), the antioxidant is oxidised and lipid hydroperoxide (LOOH) is produced in the following reaction:

\[
\text{Vitamin E} + \text{LOO}^* \rightarrow \text{Vitamin E-radical} + \text{LOOH}
\]

Lipid hydroperoxide products can react with metals such as iron to form cytotoxic products such as aldehydes, alkoxy radicals (LO*) and peroxyl radicals (LOO*):

\[
\text{LOOH} + \text{Fe}^{2+} \rightarrow \text{LO}^* + \text{Fe}^{3+} + \text{OH}^-
\]

\[
\text{LOOH} + \text{Fe}^{3+} \rightarrow \text{LOO}^* + \text{Fe}^{2+} + \text{H}^+
\]
Again, GSH-Px is required to deal with lipid hydroperoxides. Therefore selenium as an integral part of GSH-Px belongs to the first and second levels of antioxidant defence. Even the second level of antioxidant defence is not able to prevent lipid peroxidation and some biological molecules are damaged. In this case the third level of antioxidant defence deals with the repair of damaged molecules, consisting of specific enzymes such as proteases or lipases (Surai, 2002).

Whilst each level of antioxidant protection is important in its own right, it is only by ensuring they all work in harmony that complete protection can be afforded to the animal. It also ensures the efficient use of antioxidant resources that are often poorly stored in the body or in limited supply. An example is the efficient recycling of vitamin E in the presence of sufficient vitamin C and selenium.

Animal requirements for antioxidants vary with age, breed, health, work intensity, physiological status and environmental stress. Exposure to disease, air pollution (also cigarette smoke) generates oxidative stress. This makes it essential to supply adequate levels of antioxidant and precursor components for SOD, GSH-Px and catalase in forms that are readily taken up, and preferably stored in the body. When the oxidative stress level changes, tissue reserves leave the animal prepared to neutralise free radicals effectively before any serious damage occurs.

**Antioxidant sources**

Most antioxidants are delivered via the diet, either naturally occurring in raw materials or via supplementation. Fat-soluble antioxidants such as vitamin E and carotenoids operate in lipid-rich environments such as membranes, whereas water-soluble ascorbic acid (vitamin C) and glutathione, which are synthesised in the body, are found in the cell cytosol. All require adequate supplies of basic components for synthesis from dietary sources. Deficiency (and, in some cases, excess) of these elements causes oxidative stress and damage to cellular function. Selenium deficiency is a particular problem, as the main dietary sources are plant-based food ingredients that must grow in selenium-rich, alkaline soils to accumulate the element. In many parts of the world soils are very low in selenium, and intakes in both the human and animal populations have declined alarmingly in recent decades, making the need for supplementation critical.

Inorganic sources of elements such as zinc can often meet the immediate needs of the animal, but have been found to be less well retained compared to organic sources of these metals. This is because animals have evolved mechanisms to extract organic forms from the diet, which are usually bound to amino acids (Surai, 2000). An example of this is the organic selenium synthesised and stored in certain yeasts, which is primarily in the form of selenomethionine (as found in plant materials). This compound is readily absorbed and stored in the body, making it an effective source of selenium for synthesis of antioxidant enzymes during times of stress. Certain botanical ingredients have shown strong antioxidant properties, and are the focus of research in animal and human health as potential food and feed supplements. Hops, rosemary, thyme, turmeric and grapeseed extracts show interesting levels of antioxidant activity (Bagchi et al., 1998; Tucker, 2002).

**Implications of oxidative stress for companion animals**

Concerns regarding sources and effects of oxidative stress in companion animals center on cumulative effects associated with aging and age-related diseases. Cancer is a major killer of pet dogs and cats, primarily due to their long lives and exposure to oxidative stress, which can lead to the failure of correct DNA replication and tumour growth. Increases in oxidative stress can come from varying sources, and some examples are given in Table 2. The majority of work concerning the relationship between oxidation, selenium and cancer has been conducted on humans, however the mechanisms are not species-specific. An excellent review of the relationship between cancer and selenium intake showed that incidence of cancer in vulnerable subjects (human and small animal studies) can be reduced by 30-50% (Whanger, 2004).

<table>
<thead>
<tr>
<th>Internal oxidising sources</th>
<th>External oxidising sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration – mitochondria</td>
<td>Cigarette smoke</td>
</tr>
<tr>
<td>Immunity - phagocytes</td>
<td>Radiation</td>
</tr>
<tr>
<td>Reactions with transition metals (e.g. Fe)</td>
<td>UV light</td>
</tr>
<tr>
<td>Pro-oxidant inorganic elements</td>
<td>Pollution</td>
</tr>
<tr>
<td>Arachidonate pathways</td>
<td>Certain drugs</td>
</tr>
<tr>
<td>Peroxisomes</td>
<td>Chemical reagents</td>
</tr>
<tr>
<td>Under/over exercising</td>
<td>Industrial solvents</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Mycotoxins</td>
</tr>
<tr>
<td>Vaccination</td>
<td>Oxidised dietary fat</td>
</tr>
<tr>
<td></td>
<td>Disease challenges</td>
</tr>
</tbody>
</table>

Adapted from Furst, 1996.
Oxidative effects are associated with inflammatory activity. An example of this is the antioxidant extract silymarin, which has been widely demonstrated to reduce arthritic and oedemal inflammation in both humans and rodents (Figure 1). Trials were conducted with mice whereby inflammation was introduced by latex injection into the ear. The width of the resulting inflamed area was then compared between groups of mice receiving various levels of the natural antioxidant (Gupta et al., 1999). Results indicated that inflammation caused by the latex injection was reduced by nearly 30% in mice fed 50 mg/kg antioxidant or more.

Reproductive organs are also sensitive to antioxidant status. Spermatozoa are rich in polyunsaturated fatty acids and require adequate membrane protection. Therefore, if antioxidant protection is compromised, reproductive success will be compromised as well. This effect has been well documented in agricultural species such as pigs and poultry. Trials conducted in poultry (Surai, 2002) have shown important reductions in lipid peroxidation in sperm from birds fed combinations of vitamin E and organic selenium (Sel-Plex®). Figure 2 shows that peroxidation levels in sperm decreased when males were fed increasing levels of vitamin E and organic selenium.

Very young animals are particularly vulnerable to stresses that can be regulated by antioxidant enzyme precursors. Selenium reserves in muscle are released under stress conditions for the synthesis of selenoprotein enzymes such as GSH-Px to prevent damaging effects of free radical overproduction from, e.g. immune stimulation through vaccination. Body stores of such compounds are especially important since many stresses are associated with decrease in food consumption. In contrast to inorganic selenium, the selenoamino acid from organic sources fed to the dam is transferred to milk and colostrum, ensuring nursing neonates obtain a continuing supply. Trials on pigs have shown that feeding a suitable source of organic selenium (Sel-Plex®) to lactating animals can increase the levels of Se in milk, which is subsequently passed to the young. Furthermore this supplementation of selenium in the milk can then be used to generate stores in tissue such as liver. (Table 3, Figure 3).

The enzyme iodothyronine deiodinase, which is involved in thyroid hormone activation and influences basal metabolic rate and thermoregulation, is seleno-dependent. Young animals are less able to maintain body temperature, especially during disease challenge. Improving the capacity for tissue retention of selenium could potentially improve neonate livability.

### Table 3. Selenium content of tissues of neonatal piglets from sows receiving different selenium supplements.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Inorganic Se</th>
<th>Sel-Plex®</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se, ppm</td>
<td>0</td>
<td>0.15</td>
<td>0.3</td>
<td>0.15</td>
</tr>
<tr>
<td>Liver, mg</td>
<td>0.197</td>
<td>0.238</td>
<td>0.348</td>
<td>0.348</td>
</tr>
<tr>
<td>Muscle, mg</td>
<td>0.037</td>
<td>0.046</td>
<td>0.066</td>
<td>0.066</td>
</tr>
<tr>
<td>Total, mg</td>
<td>0.075</td>
<td>0.079</td>
<td>0.123</td>
<td>0.123</td>
</tr>
</tbody>
</table>

Mahan, 2002.
A major use of antioxidants, especially those of a chemical nature, is as a food preservative to increase shelflife and ensure palatability. Recently there has been increasing interest in using more natural forms of antioxidants in pet foods – both as a marketing opportunity and to allay consumer worries regarding the use of chemicals. The established use of meat and animal by-products in formulations, as well as the inclusion of polyunsaturated fats in premium pet foods, require specialised antioxidant programs to ensure food quality. The high levels of processing experienced, especially in extruded or canned foods, increase exposure to heat and air, promoting autooxidation reactions and facilitating rancidity. Any

**Stability of pet food**

![Figure 2. Effect of Sel-Plex® and vitamin E supplementation on fresh sperm peroxidation (adapted from Surai, 2002).](image)

![Figure 3. Effect of selenium level (ppm) and form on sow milk selenium content (Mahan, 2002).](image)
antioxidant, whether chemical, tocopherol-based or derived from natural plant sources, must be stable at high temperatures to be practical under such extreme conditions.

Losses of natural antioxidants from extrusion are frequently reported as being 50% or higher, making heat tolerance a key attribute for natural alternatives replacing synthetics. Research at the University of Santiago in Chile (Valenzuela, 2003) demonstrated that a commercial antioxidant system containing natural mixed tocopherols and rosemary extract (Nature Ban™) was thermally stable for 1 hr at 200°C and for 2 hrs at 150°C when tested on refined sardine oil.

Commercial experience indicates that certain natural antioxidants show less than 30% losses after extrusion. Figure 4 shows the comparative stability of chemical and natural antioxidants, including the commercial product Nature Ban™. Natural products show similar levels of protection as the chemical products when applied at similar doses. However, a combination of natural antioxidants, as supplied via Nature Ban™, gave the longest stability period.

Comparisons between chemical and natural antioxidants for preventing rancidity in animal fats have also been conducted. Prevention of peroxide generation was monitored in chicken fat samples treated with chemical or natural antioxidants (Valenzuela, 2002). The results clearly showed that the combination of natural antioxidants in Nature Ban™ resulted in the lowest peroxide values over an extended storage period at high temperature. Such results demonstrate that the use of natural alternatives can ensure both the quality of pet food and the demands for more natural ingredients by purchasers.

**Branding antioxidant benefits**

Branding and marketing pet food typically follows the same consumer decisions as for human food. Recent trend analysis in retailing has shown that the growth market is for high quality and added value food (Hughes, 2003), so opportunities also exist for higher quality pet food formulated with functional ingredients such as antioxidants. As people realise the importance of antioxidants in their own diets, they increasingly look for similar benefits for their pets. An increase in public awareness regarding the importance of antioxidant-containing foods as well as mineral supplementation and ‘eating for health’ is already responsible for changes in many buying decisions.

A key factor in the marketing of antioxidant-supplemented pet foods is effective communication with the person making the purchase. The plethora of pet foods available is often bewildering, and, while it is well established that people are willing to pay...
for perceived higher quality, it is essential to put the message across regarding the added value antioxidants bring to the diet. This should be done in conjunction with making a bold statement via packaging that antioxidants are included. Some manufacturers make claims for antioxidants based solely on the standard practise of adding vitamin E to the diet. Hence it is of key importance to use and promote the explicit benefits of well-established antioxidant products that add health and welfare benefits, backed up with proven science.

References


A changing landscape: the pet food market in Europe

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Introduction

The European pet food market has continuously expanded during recent decades. This can be explained by a long lasting growth in pet ownership, by the decreasing use of table scraps and the general trend toward convenience foods. In the past years the situation in Western Europe has changed and the growth rate of the market has slowed down in many countries so that a mature market situation has developed. The market split into different segments. Pet owners’ demands for premium products and sophisticated accessories for their animals have led to an increase of sales in those sectors. On the other hand, it is obvious that other pet owners have a requirement for pet foods that fulfil the nutritional needs of the animal at reasonable prices. This trend has been strengthened by the unfavourable economic situation of the last years. Another important aspect for the pet food market is the fact that the European Community has expanded and will expand in the future. The applicant countries offer promising opportunities due to the lower level of calorie coverage by prepared pet food and the expected growth of the markets. But even in the old member states of the European Union there exist considerable differences among the countries. Although the standard of living in those countries is more or less comparable, there are obvious differences in the habits of keeping pets and the traditions of pet feeding that affect the market. Europe is not uniform. The European countries differ in population density of dogs and cats, the calorie coverage by commercial pet foods and the balance between commercial foods and the traditional ways to feed pets with table scraps.

The European pet population in 2004

The number of households with pets is estimated by the European Pet Food Industry Federation (FEDIAF) to have reached 55 million. The number of cats is higher than the number of dogs; and estimates are that 47 million cats and 41 million dogs are kept in the Western European countries (FEDIAF, 2004). A population of 35 million pet birds, 9 million aquaria and 36 million other pets, mainly rodents and small mammals, but also reptiles, must be considered as another important segment of the market. Over the last years, certain trends have been observed. The number of dogs tends to be stable or in some countries slightly decreasing while the number of cats is increasing. Based on a total number of households in Western Europe of 155.6 million, it can be estimated that 21% keep dogs and 20% keep cats with an average number of 1.1 and 1.4 dogs and cats, respectively (FEDIAF, 2004). The number of cats has increased and the trend to keep more than one cat per household is observed in many countries (ZZF, 2001). This trend reflects the popularity of cats as a ‘lifestyle adapted’ pet, and may also be explained by the recommendations to facilitate socialisation by keeping more than one cat, especially in households where cats must stay alone over longer times during the day. The trend for the number of dogs to decrease can be explained by the fact that lifestyles of pet owners have changed. In addition, the selection of dogs as pets has also been subject to other influences. External factors have had great impact on the public opinion towards the role of dogs as pets. In recent years it has become obvious that keeping dogs as pets is a very sensitive topic for the public. Discussions have come up on the role of
dogs in urban environments regarding problems for public hygiene. Additional controversies developed after some unfortunate accidents with dogs that induced a negative picture of ‘dangerous’ dogs and dog-keeping in general in the media. For instance, in Germany a discussion along these lines developed after a series of bite attacks by dogs was picked up by the media including one severe accident that killed a young child. This unfortunate event focused public opinion transiently but strongly against ‘aggressive’ and ‘dangerous’ dogs and consequently induced a general discussion on dog keeping. This had a negative impact on the number of dogs, as documented by the statistical data (Table 1) of the German Kennel Club (VDH, 2003). These figures show the trend of the dog population in Germany, which is one of the five countries (together with France, the United Kingdom, Italy and Spain) in Western Europe with strong economic impact on the feed industry.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of puppies</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>90257</td>
</tr>
<tr>
<td>2001</td>
<td>89822</td>
</tr>
<tr>
<td>2000</td>
<td>88659</td>
</tr>
<tr>
<td>1999</td>
<td>97860</td>
</tr>
<tr>
<td>1998</td>
<td>106815</td>
</tr>
<tr>
<td>1997</td>
<td>114670</td>
</tr>
<tr>
<td>1996</td>
<td>117893</td>
</tr>
<tr>
<td>1995</td>
<td>119460</td>
</tr>
<tr>
<td>1994</td>
<td>114690</td>
</tr>
<tr>
<td>1993</td>
<td>109785</td>
</tr>
<tr>
<td>1992</td>
<td>106490</td>
</tr>
</tbody>
</table>

VDH, 2003; 2004

The stable or negative trend in the dog population is also observed with regard to dog breed and dog size preferences. In Europe the number of small sized dogs (<10 kg BW) was estimated to be 34%, the medium and large sized dogs make up 37 and 29% of the total population (Euromonitor, 2001). The southern countries (France, Italy, Spain, Portugal) have a certain preference for smaller dogs, while other - especially the northern - countries seem to prefer larger breeds. This preference is seen in Germany, Belgium, Netherlands, Denmark, Norway, Sweden, Austria, and Switzerland. However, the number of large dog breeds is decreasing and a shift in the dog population can be expected towards smaller breeds in many of these countries.

Regarding the age structure of dogs in Western Europe, it is estimated that 9% of the dogs are younger than two years and 16% older than 10 years. The number of older animals in the United Kingdom (23%), the Netherlands (21%), France (18%), and Germany (17%) is above the European average. Lower numbers are seen in Portugal (7%), Norway (11%), Italy and Spain (12%) (Euromonitor, 2001).

In contrast to the dog population the number of cats has increased steadily in recent years. This development has huge economic consequences for the related industries. Data on the total cat population in Europe differ, but in most countries of Western Europe the number of cats is considered to be either stable or increasing. France, United Kingdom, Italy and Germany are the countries with the biggest cat populations (Euromonitor, 2001). Regarding the number of cats per household, differences are obvious. The estimated average number of cats in Western Europe is 1.4 per household. Households with more than one cat are seen more frequently in Austria (2.2), Switzerland (1.8), Belgium (1.8), Norway (1.8), Spain (1.7), the United Kingdom (1.6), and Italy (1.6). Regarding the age of domestic cats, 26% of the total cat population is younger than two years and 18% is older than 10 years. Countries with a higher population of older cats are the United Kingdom (29%), the Netherlands (23%), Germany (22%), Sweden (21%), and Switzerland (19%).

The situation in the application countries and in Eastern Europe is difficult to predict because no statistical data have been documented in the public literature. Assuming that the density of dogs and cats is comparable to Western Europe, it can be expected that these countries offer a dynamic and promising area for future economic activities. The markets for pet food and care products are relatively undeveloped and the use of prepared pet food is much lower compared to the Western European countries.

Demographic and social factors

Several facts must be taken into consideration when speculating about the future of the European pet food market. The current population dynamics in Europe show negative trends in many countries. Birth rates have slowed or are decreasing, and the median age of the population is increasing. In many countries a trend toward urbanisation with higher numbers of households and lower numbers of persons per
household is observed. In addition, lifestyles have changed. Individualization is important for many young people, and the length of time a person is out of the house over the day or for longer periods has increased. Traditionally, mobility has been lower in many countries of the European Union compared to other parts of the world, but due to the changes in economic conditions and lifestyles of young people, it has increased in recent years. Statistical data are available to support these assumptions. Taking figures for Germany as an example (ZZF, 2001), most pets are kept by owners aged 35-49 years (39% of all pets) with a clear relation to the number of family members. Pets are often kept in families with at least one child (46% of all pets), lower frequencies are found in single households (22%) and with couples without children (32%). Based on a recent statistical evaluation in Germany (ZZF, 2003) with 30,500 interviews, this trend must be interpreted in light of some new aspects. According to this investigation, couples without children and singles represent on a household basis 60% of cat owners, 59% of dog owners, 58% of the pet bird owners and 48% of the aquarium owners. Singles seem to prefer cats as pets, dogs are only second choice. This fact is important, because in Germany and in other countries it can be expected that the number of households, either singles or couples without children, will increase and will make up about 72% of the total number of households in 2010. It can be expected that the number of cats will be increasing in the next years because cats are more suitable for this household structure than are dogs. The population of dogs, pet birds, and also of small mammals, which is currently still expanding, will probably decrease.

These important demographic trends are comparable in many other member states. On the other hand, changing lifestyles of pet owners have other effects on the development of pet markets. Importantly, these demographic changes also affect the position of the animal in society. Humanisation of pets is obvious, and can be explained by the demographic changes bringing pets into a specific role as social partners. This development has been noted and further supported by the marketing strategies of pet food and supplier companies. Seeing pets as beings with equal or at least similar requirements as humans means that many owners are willing to spend more time and money to indulge their animals.

Pet food industry and market expectations

The market for pet food and related products is mature in most Western European countries. Volumes of dog food are falling in several countries, inducing a general fall of pet food volumes, but not necessarily on a sales basis. FEDIAF estimates the total number of pet food companies in Europe at 450 and the current volume of all pet food is estimated at 5 million tons with an estimated sales value of 8.5 billion € (FEDIAF, 2004). The growth rate of the pet food industry was 3% over the last three years (2001-2003), which is a reasonably good result compared to stagnation or negative growth rates in other industries. The number of employees in the pet food industry was 21,000 persons working directly in the industry. Another 30,000 persons are estimated to work indirectly for this industry. The significance of the pet food industry for the total agricultural sector is becoming increasingly important. The yearly purchase of agricultural by-products in the European Union was 2.75 million tons (FEDIAF, 2004). Manufacturers will have to continue to be innovative, because innovation will be an important feature of successful companies. However, in recent years moderate increases in quantity have been accompanied by falling returns in the market for ready-mixed pet food (Siessegger, 1997). The distribution and retail channels have changed significantly in the past years. Traditionally, pet food was either sold in grocery stores or in many European countries by small, traditionally structured ‘over-the-counter shops’. Many of these offered a good standard of products and had a specific strength in advising customers. On the other hand, small shops have lost significant market share all over Europe due to the competitive price situation. The relative significance of grocery stores and specialised pet food markets has increased. The small solitary shops have and will probably maintain a certain niche with higher priced products and with products that need more explanation for the consumers. Another specific target group for this sector is non-experienced owners that need more continuous advice. While the volume of pet food has reached a certain plateau in Europe, the situation in the individual member states differs. It has become obvious that customer demands are increasingly orientated toward premium products, treats, and in the segment of complete diets toward dry food in dogs (Figure 1). Similar trends can be observed for cats.
Consumer expectations

Pet owners have different and specific expectations that pet food should fulfil. Consumers have a demand for high quality standards in each price segment. Safety, nutritional adequacy and health promoting effects of pet food products are an important issue. ‘Healthiness’ is a major aspect for many consumers, especially those who are prepared to pay higher prices for premium products. Another group of consumers do not consider health aspects a priority, but they expect that the pet enjoys eating a specific product or that they themselves are enjoying feeding the specific product to their pet. A third group of pet owners is mainly interested in getting access to reasonable products at economic prices. All will expect not only high nutritional value and adequacy, but also high palatability and digestibility of the food. In many of the western European countries issues have been raised around the ethical aspects of pet food. Since the BSE crisis certain raw materials are no longer used by the pet food industry and have been discarded from the pet food chain earlier than from the human food chain. The ingredients of animal origin must be approved by the veterinary service and must be fit for human consumption, otherwise they cannot be used for pet food production. In the higher price segment it is expected by consumers that manufacturers conduct additional controls in their plants to assure optimum quality of the ingredients. Future developments are difficult to predict, but it can be assumed that the trends of the last five years will continue: a considerable and growing part of the consumers will demand premium quality and will be prepared to pay adequate prices. Quality, safety and nutritional adequacy will be an ongoing issue for this group. Functionality of pet food is debated and will result in new products, but in this aspect the European perspective seems to differ from other parts of the world. In Europe, the use of dietary supplements is less common in humans and therefore consumers are less willing to use functional supplements for pets. On the other hand, many benefits of functional pet foods will be appreciated: palatable treats with specific functionality, products with proven efficacy in the promotion of well-being, health and longevity, and products of natural origin.

References


Figure 1. Market trends of moist, semi-moist, dry pet food, mixers and treats for dogs in the United Kingdom (PFMA, 2004).


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Can we re-invent feeding animals? Feed companies? Ingredients and nutrient supplies? Can we change how food animal products and crops are perceived by, and marketed to, consumers?

The theme of the 20th Annual Alltech Symposium focuses on doing exactly that: re-defining how we feed animals and re-imagining our agribusinesses to create the compelling force behind an exciting future.

The basic and applied research is done. Natural feeding strategies can reduce environmental impact, replace antibiotics, make producers more competitive and change how consumers view and value food animal products. There is a shift in the dynamic of the industry as well. Change is no longer something ‘happening to’ the feed industry. Change is something the industry makes happen.