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S E R V I C E

# 63rd Minnesota Nutrition Conference & Pre-Conference Symposium: Rendering: A Foundation for Food Security

September 17-18, 2002

Eagan, Minnesota



# 63rd Minnesota Nutrition Conference

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The Minnesota Nutrition Conference is recognized as an important forum for the nutrition industry. Local, national and international animal nutritionists gather to learn about the current, innovative research being conducted at universities, in industry and at government centers. It is attended by people like you, nutritionists, industry representatives, veterinarians, educators and producers who need to stay ahead in the fast changing field of animal nutrition.

This year's conference features dairy, beef, swine and poultry nutrition.

The conference will be preceded by the pre-conference symposium, **Rendering: A Foundation for Food Security**, on Tuesday, September 17, 2002.

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## TUESDAY SEPTEMBER 17, 2002

63rd Minnesota Nutrition Conference

### Pre-Conference Symposium: Rendering: A Foundation for Food Security

8:00 Registration - Coffee and Danish

8:20 Welcome and Introduction - *Don Franco*, President, APPI

**SESSION I: BIOSECURITY OF ANIMAL BY-PRODUCTS** - *Ross Hamilton*, Moderator

8:30 **Rendered Products - Secure from Food Pathogens**

*Fred Troutt*, University of Illinois

9:00 **How Important is Salmonella Control in Feed?**

*Peter R. Davies*, Massey University

9:30 **The Harvard Risk Analysis - the United States has Minimal Risk of TSE's**

George Gray, Harvard School of Public Health

10:15 Break

**SESSION II: FINISHED PRODUCTS - IMPROVED NUTRIENT VALUE** - *David Kirstein*, Moderator

10:30 **Protein/Amino Acid Digestibility** - *Carl Parsons*, University of Illinois

11:00 **Fat Utilization and Energy Prediction**, *Julian Wiseman*, University of Nottingham

11:30 **Realizing the Value of Animal Proteins in Commercial Formulations**

*Roy Brister*, Tyson Foods

12:00 Discussion - Questions and Answers

12:15 Lunch - Sponsored by APPI and Central Region Renderers Association

### GENERAL SESSION - PHOSPHORUS SYMPOSIUM

1:00 **Phosphorus and Animal Manures from a Soil Scientist's Perspective**

*Neil Hansen*, University of Minnesota

1:45 **Meeting Phosphorus Requirements of Ruminants in an Environmentally Responsible Way** - *Larry Satter*, USDA-ARS, Madison, Wisconsin

2:30 Break - Sponsored by Gold & Silver Sponsors

3:00 **Approaches to Meeting the Nonruminant Animal's Phosphorus Requirement**

*Gary Cromwell*, University of Kentucky *Sponsored by Hubbard Feeds, Inc.*

3:40 **Establishing National Standards for Estimating Nutrient Excretion from Livestock**

*Wendy Powers*, Iowa State University

4:15 Speaker Panel

4:45 Adjourn

5:00-6:30 Reception with complimentary hors d'oeuvres *sponsored by Northwest Feed*

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## WEDNESDAY SEPTEMBER 18, 2002

### 63rd Minnesota Nutrition Conference

#### Ruminant Session

- 8:00 Registration - Coffee and Pastries
- 9:00 **Predicting Feed Intake of Lactating Cows** - *Dana Allen*, University of Minnesota
- 9:40 **Evaluation of the 2001 Dairy NRC Protein and Energy Requirements** - *Jim Linn*, University of Minnesota
- 10:20 Break - Sponsored by Gold & Silver Sponsors
- 10:50 **Evaluating the Energy Content of Fat Supplements in Ruminant Diets** - *Rick Grummer*, University of Wisconsin
- 11:30 Panel discussion with morning speakers
- 12:15 Lunch - *Sponsored by Diamond V Mills*
- 1:15 **Use of Blood Parameters Related to Nutrition of Dairy Cows**, *Tom Herdt*, Michigan State University
- 1:55 **Implant Strategies for Dairy Steers** - *Hugh Chester-Jones*, University of Minnesota
- 2:30 Break - Sponsored by Gold & Silver Sponsors
- 3:00 **Receiving Diets for Feedlot Cattle** - *Francis Fluharty*, Ohio State University
- 3:40 **Corn Processing for Ruminants** - *Galen Erickson*, University of Nebraska
- 4:15 Panel discussion with afternoon speakers
- 4:45 Adjourn

#### Nonruminant Session

- 8:00 Registration - Coffee and Pastries
- 9:00 **Use of Oligosaccharides and Gut Modifiers as Replacements for Dietary Antibiotics**, *Peter Ferket*, North Carolina State University
- 9:40 **Nutrition for Alternative Poultry Production** - *Jacque Jacob*, University of Minnesota
- 10:20 Break - Sponsored by Gold & Silver Sponsors
- 10:50 **Factors Affecting Feed Intake of Meat Birds** - *Peter Ferket*, North Carolina State University
- 11:30 Panel discussion with morning speakers
- 12:15 Lunch - Sponsored by Diamond V Mills
- 1:15 **Exogenous Enzymes for Pig and Poultry Feed Ingredients** - *Julian Wiseman*, University of Nottingham
- 1:55 **Fiber Nutrition of Sows**, *Lee Johnston*, University of Minnesota
- 2:30 Break - Sponsored by Gold & Silver Sponsors
- 3:00 **Insulin in Swine Diets**, *Sam Baidoo*, University of Minnesota
- 3:40 **Relationship Between DDGS and Ileitis in Swine** - *Mark Whitney*, University of Minnesota
- 4:15 Panel discussion with afternoon speakers
- 4:45 Adjourn

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FASS Congressional Fellow  
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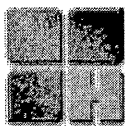
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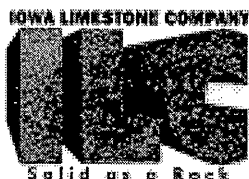
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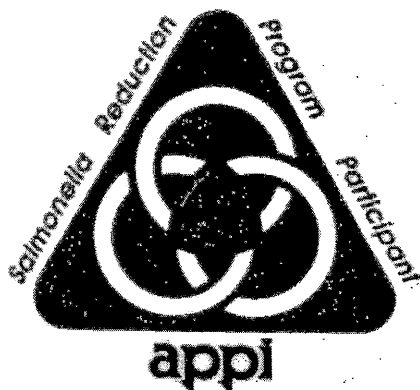
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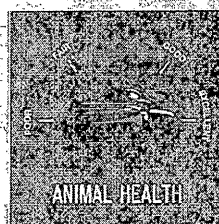
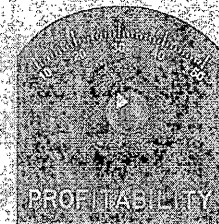
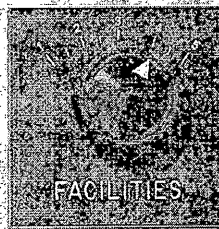
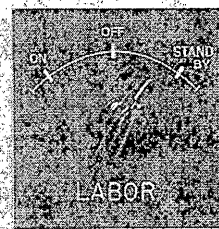
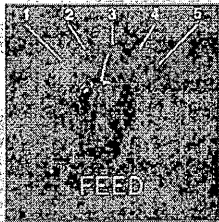
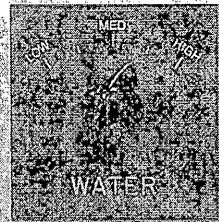
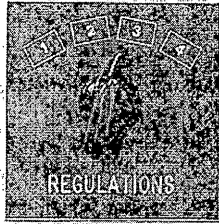
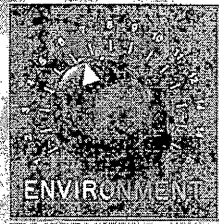
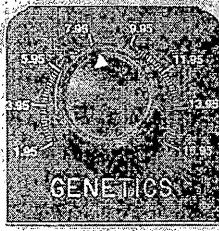
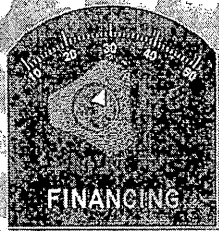
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\* Papers appear in the order of presentation at the 63rd Minnesota Nutrition Conference & Pre-Conference Symposium held September 17-18, 2002.

# PREVALENCE OF SELECTED FOODBORNE PATHOGENS IN RENDERED PRODUCTS

(Abstract)

H. Fred Troutt, VMD, PhD  
David Schaeffer, PhD  
Leslie Deem, MS  
Ibulaimu Kakoma, DVM, MS  
University of Illinois, Urbana

There has been national concern about foodborne illness and the safety of both animal and human foods of animal origin. This concern has stimulated animal commodity organizations, meat processors, animal feed manufacturers and animal feed ingredient suppliers to establish quality assurance programs that usually conform to Hazard Analysis Critical Control Point (HACCP) efforts. Indeed, the rendering industry has widely formulated HACCP-based programs as an element of quality assurance in fostering sanitation and hygiene in the production of rendered animal byproducts.

The national concern for the transmission of foodborne organisms from food animals and to assess possible relevant biosecurity hazards in the rendering process prompted us to carry out a pilot study at 17 rendering facilities in the Midwestern United States. Samples were obtained during summer and winter. This study focused on the microbiological examination of three specimen types from each facility for the presence of *Listeria monocytogenes*, *Campylobacter jejuni*, *Clostridium perfringens*, *Salmonella spp* and the presence in selected specimens of *Escherichia coli*. The specimen types examined from each facility were: raw material obtained just prior to entry into the cooker, the material resulting from the cooking-expelling process, "crax," and the final rendered product (FRP) from each facility.

In addition to the microbiological assessments, water activity, pH and proximate analysis measures were conducted on the final rendered products.

*Clostridium perfringens*, *Listeria monocytogenes*, *Campylobacter jejuni* and *Salmonella spp* were isolated from raw materials. *Clostridium perfringens*, *Listeria monocytogenes* and *Salmonella spp* were not isolated from the "crax" samples. However, *Salmonella spp* were isolated from the final rendered products (9 of 42 in winter; 13 of 42 in summer).

As expected, in general, the raw material used in the rendering process was heavily contaminated with the index potential foodborne pathogens. We did not isolate the index organisms from the "crax" samples, indicating these organisms are inactivated by the rendering process.

# HOW IMPORTANT IS CONTROLLING *SALMONELLA* CONTAMINATION OF FEED?

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## INTRODUCTION

The complexity of the ecology and epidemiology of *Salmonella* is difficult to overstate. The annual burden of non-typhoidal human salmonellosis in the USA was recently estimated to be of the order of 1.5 million cases (including over 580 deaths), and 95% of cases were attributed to foodborne infection (Mead et al., 1999). The position of *Salmonella* as the premier foodborne hazard associated with pork was consolidated by two events in the mid 1990's:

- The HACCP/Pathogen Reduction Act ("Mega-Reg") in the USA in 1996, which saw the implementation of microbiological monitoring for *Salmonella* at slaughter plants
- The implementation of a national *Salmonella* control program by the Danish swine industry (Mousing et al., 1997)

The ultimate aim of controlling foodborne hazards is to reduce the risk of disease to consumers, and thereby the considerable economic burden of foodborne illnesses. For most microbial hazards, the question of how best to allocate limited resources to optimize control remains problematic. The 'farm to table' concept (acknowledging that all participants in the continuum of food production and consumption bear some responsibility for reducing the risk of foodborne disease) has emerged as the fashionable paradigm for food safety. However, the high level of consensus about the appropriateness of a 'farm-to-table' approach has yet to be matched by meaningful analysis of how this should be achieved. In particular, generic calls for control of foodborne pathogens in food animal populations have somewhat naively presumed that such an approach is feasible and efficacious, and have rarely touched on economic constraints.

## PREHARVEST FOOD SAFETY IN THE CONTEXT OF RISK MANAGEMENT

Crudely, we can divide the food supply continuum into sectors of production, processing, distribution, and consumption. Within each sector, a range of interventions might be applied to reduce risk of foodborne hazards, and the goal is to define the optimal mix of interventions across the continuum that delivers maximum risk reduction at minimal cost. Ideally, interventions should be applied strategically at those points where the greatest impact on ultimate risk will be achieved. However, participants in all sectors should be motivated by their own self interest to ensure that they take what measures they can to reduce risk. Public education should reinforce that the food supply will never be risk free, and that appropriate kitchen hygiene and cooking practices are powerful tools for mitigating risk of multiple biological hazards. Processors want to be meticulous in development and implementation of good management practices and process control procedures, as brand image can be destroyed almost overnight by adverse events, be they disease outbreaks or product recalls. Companies must minimally meet

regulatory goals for microbiological safety, but for commercial or other reasons may aspire to standards that exceed national or international benchmarks. Animal producers are ethically bound to take what steps they can to reduce foodborne hazards in animals under their care. However, potential preharvest interventions to control *Salmonella*, or other foodborne hazards, need to be considered in terms of cost and impact.

In the context of reducing risk of foodborne disease to consumers, the value of any given intervention is determined by:

- 1) Efficacy (including spectrum of hazards controlled)
- 2) Cost
- 3) Probability of post-intervention contamination

The ideal intervention would have low cost, be effective against all significant hazards, and be implemented near to the point of consumption (minimising the probability of subsequent contamination). For biological hazards with relatively complex ecologies, it is unrealistic to expect single interventions (particularly far 'upstream' from consumers) to provide adequate protection. Preharvest control holds most promise for hazards that have relatively simple epidemiology (i.e., limited modes of transmission), including minimal risk of cross-contamination and inability to replicate in products, and most specifically for hazards with negligible potential for 'downstream' contamination. The risk of post-farm contamination may be significant for several key bacterial foodborne pathogens of swine. A microbiological study of the pork supply chain in Korea indicated that the cold chain system for transporting and merchandising of pork products was deficient, and this sector appears to be a major contributor to risk of contamination of product offered to consumers in that country (Rho et al., 2001).

Producers, and their suppliers, bear the major responsibility for risk minimisation for chemical or physical hazards. The recent European disasters with dioxin and medroxyprogesterone acetate highlight the importance of adequate quality control systems in the supply of animal feeds. Due to the relatively simple biology of *Trichinella spiralis* and *Toxoplasma gondii*, simple preharvest measures have led to marked reduction in the prevalence of these meatborne parasites in swine in the USA (Davies et al., 1998). In contrast, in the light of current epidemiologic knowledge, availability of effective interventions, and probability of downstream contamination, it is hard to make a strong case for preharvest control of *Campylobacter* or *Listeria* (and also *Yersinia*) in swine production. Collectively, preharvest interventions to control enteric foodborne pathogens in pigs are characterised by uncertain efficacy, uncertain costs, and (by definition) a relatively high probability of post-intervention contamination.

### WHAT ABOUT SALMONELLA?

The technical feasibility of preharvest control of *Salmonella* using microbiological testing and regulation has been demonstrated by the Swedish poultry and swine industries (Wierup 1997). However, perhaps the most eloquent statement of the difficulty and cost of implementing the 'Swedish model' for *Salmonella* control is that, despite its apparent success, after some 40 years it has not been adopted by any major swine or poultry producing nation. The push for preharvest control of *Salmonella* gained considerable momentum from the implementation of the Danish

*Salmonella* control program, which has focused on preharvest interventions in herds identified serologically to present the highest infection risks. The concept of preharvest food safety assumes management decisions can be taken that will to reduce exposure of animals to potential hazards, thereby ultimately reducing risk to consumers. There are 2 general approaches for preharvest control of foodborne pathogens:

- Establishment and maintenance of pathogen-free animal populations (Swedish model)
- Reduction of prevalence in endemically infected herds through improved production systems and management (Danish model)

To assess the relative merits of these approaches, one must understand the principal sources of infection for herds, risk factors for spread of infection within infected populations, and the feasibility of control measures. As stated previously, selection of interventions should consider efficacy, cost and probability of downstream contamination. Although it is evident from the Danish experience that some progress is achievable using management interventions to reduce rather than exclude *Salmonella* on farms, the efficacy and cost of specific interventions is not well understood. This program has required an annual investment of the order of \$14 million per annum (or approximately \$0.70 per pig marketed), and a modeling approach was recently used to gain some insight into the question of future resource allocation for *Salmonella* control in the Danish swine industry (Alban and Staerk, 2002). The model predicted that improvement of any single factor had a limited impact on the level of contamination, and the largest reduction was observed when several factors were improved concurrently. As one would expect, lack of data was identified as a major limitation to the model.

In the USA it appears that a high probability of downstream infection, particularly in lairages, is a major concern with respect to preharvest control of *Salmonella*, at least at the individual farm level (Davies et al., 1999; Hurd et al., 2002). Similarly, recent studies in Holland found that holding in lairage for 2 hours or more led to substantial risk of *Salmonella* infection of pigs (Swanenburg et al., 2001), while data from Denmark suggest that cross-contamination at lairage may be less important in that country (Boes et al, 2001). However, the data in the USA indicate that preharvest efforts to control on individual farms is likely to be unrewarding (in terms of ultimate risk reduction for consumers) unless separate slaughter is possible for low risk and high risk populations (as suggested in Holland and practised in Denmark). Claims of food safety assurance founded on preharvest procedures for *Salmonella* are highly questionable unless separate slaughter is also conducted. Developing interventions targeting the immediate preslaughter management should clearly be a priority in the US swine industry. Alternatively, preharvest measures would need to be applied across the population of herds supplying a given processing plant. Control of *Salmonella* contamination of feed would be an integral component of such an approach.

#### HOW IMPORTANT IS SALMONELLA CONTROL IN FEED?

The animal-feed/animal-infection/human-infection axis is an historically important and well-documented facet of the epidemiology of *Salmonella*. Control of *Salmonella* in animal feed has been a major focus of *Salmonella* control efforts internationally for several decades, but feed mills or feed are still implicated as important sources of *Salmonella* in animal production,

particularly in poultry (Corry et al., 2002). Despite decades of effort to reduce *Salmonella* in animal feed, and apparently low levels of *Salmonella* in feed in many countries, overall success of 'feed oriented' control has not been spectacular (if measured in terms of prevalence of animal infection or incidence of human disease). In intensive studies of multiple-site systems in North Carolina (Davies et al., 1997; Funk et al., 2001) prevalence of *Salmonella* shedding by pigs was common (over 50% of animals in some herds) although *Salmonella* were rarely isolated from feed (e.g., 0.25% of 800 samples in the latter study). While feeds of animal origin have received the most attention as sources of *Salmonella*, it is now well recognized that feeds of plant origin, such as soybean meal, are often contaminated with *Salmonella*. Arguably, all raw feed components should be regarded as potentially contaminated. It is therefore not surprising that wide diversity of *Salmonella* serovars is typically reported in surveys of feed contamination (Davies and Wray, 1997; Harris et al., 1997). Complete exclusion of contaminated raw materials is impractical, hence appropriate process control and decontamination steps in feed mills are essential to avoid dissemination of contaminated feed to herds. An English study found contamination of ingredient intake pits and outloading gantries for finished products in feed mills by wild bird droppings containing *Salmonella* (Davies and Wray, 1997). This underlines the high degree of difficulty in providing feed that is completely *Salmonella* free.

Without disputing the established role of feed as a potential source of *Salmonella*, there are some grounds on which to question the central role traditionally afforded to feed. Most notably, the serovars of *Salmonella* isolated from feed are frequently not those found most commonly in animal populations nor human cases (Murray 1994; Berends et al., 1996; Veldman et al., 1995). More specifically, the serovars of overriding current human health significance (*S. Enteritidis* and *S. Typhimurium*) are relatively uncommon in feed (Bisping 1993; Stege et al., 1997; McIlroy 1998). This raises an important question that has considerable implications for control of *Salmonella* in livestock and the role of feed - should all *Salmonella* serovars receive equal emphasis in control programs? Although *Salmonella* are geographically ubiquitous, the predominating serovars vary both temporally and geographically. Most medical bacteriologists and clinicians retain the conventional view that all *Salmonella* are pathogens, despite the fact that relatively few serovars are responsible for most human and animal disease. While *S. Typhimurium*, *S. Enteritidis*, *S. Infantis* and *S. heidelberg* are regularly ranked among the most frequent isolates from humans and animals in many countries (D'aoust 1989) the vast majority of serovars appear to be of minimal epidemiologic importance (Joseph 1988). Wierup (1997) suggested that *Salmonella* surveillance should encompass all *Salmonella* but control could be focused on serovars responsible for most disease. Inadequate attention has been given to the possibility that the biology of *Salmonella* serovars may be quite diverse. The profile of serovars isolated from feed often differs from that found in animals consuming the feed (Funk et al, 2001), and some serovars occurring in feed may not become established in pigs (van Winsen et al., 2001). It is possible that a considerable proportion of *Salmonella* serovars that can be isolated from feed present very little risk of infection to pigs or humans.

The appropriate emphasis to give to controlling *Salmonella* in feed should be primarily determined by the goals of the overall control program. Contamination of feed is a greater focus of attention in the Swedish control programs, where the objective is exclusion of *Salmonella* from herds. In contrast, controlling *Salmonella* contamination in feed is a lesser (though still important) component of the Danish program, where the objective is reduction of *Salmonella*

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# EVALUATING THE POTENTIAL FOR SPREAD OF BSE IN THE UNITED STATES

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## BACKGROUND

In 1998 the United States Department of Agriculture asked the Harvard Center for Risk Analysis to evaluate the robustness of U.S. measures to prevent the spread of bovine spongiform encephalopathy (BSE or "mad cow disease") to animals and humans if it were to arise in this country. BSE is a member of a family of diseases that includes scrapie in sheep and goats, chronic wasting disease in certain North American deer and elk, transmissible mink encephalopathy, and the human ailments Creutzfeldt-Jakob disease, variant Creutzfeldt-Jakob disease, and Kuru.

BSE is not known to exist in the United States. Understanding of the ways in which it could spread if introduced can be helpful in evaluating current risk management strategies and evaluating the need for further measures. We have therefore developed a probabilistic simulation model to help characterize the consequences of introducing BSE into the U.S. Our model allows us to predict, for example, the number of newly infected animals that would result from the introduction of BSE, the time course of the disease following its introduction, and the potential for human exposure to infectious tissues. We can also evaluate key processes and procedures that make the spread of disease more or less likely. Results from the simulation are presented as probability distributions reflecting the probabilistic nature of the model and the processes simulated.

The largest outbreak of BSE occurred in the UK during the late 1980s and early 1990s. Our model relies upon the currently accepted theory of the spread of BSE in the UK. Although the agent responsible for BSE has not been conclusively identified, the leading hypothesis suggests that it is caused by an abnormally configured prion protein that is normally found in the host. Although the origin of the disease in the UK is not clear, it appears that the recycling of rendered cattle tissue as cattle feed was responsible for its rapid spread. In particular, scientists believe that when infected animals were slaughtered, those parts not used for human food were rendered (*i.e.*, ground up and cooked) and ultimately used as protein supplements in cattle feed. Because of the resilience of the abnormally folded prion, a sufficient portion often survived this processing and ultimately infected those cattle consuming this feed. Our model reflects the assumption that the recycling of rendered cattle tissue as feed provides a means by which BSE can be spread from one animal to another, although we also allow for the possibility that the disease can also be spread directly from an infected female to her offspring.

The simulation model can be thought of as having five components, (see Cohen *et al.* for a more detailed discussion of the model). The first component characterizes the life cycle of cattle in the U.S., including the rates at which they are born, slaughtered, become infected with BSE, or die of natural causes unrelated to BSE. The second component of the model describes how animals sent to slaughter are processed. Tissue may be disposed of, sent to rendering, or prepared for potential human consumption. The third component of the model characterizes the disposition of material sent to rendering. That material may be diverted to uses that eliminate the possibility of exposing domestic cattle or the U.S. population (*e.g.*, because it will be disposed of, exported, or used to produce feed for animals other than cattle) or end up in feed that is administered to cattle. The fourth component of the model quantifies infectivity in material presented for potential human consumption. The final component quantifies the proportion of cattle that die prior to being sent to slaughter that are rendered.

## RESULTS

Our analysis finds that the current US system is quite resistant to any challenge from BSE or a similar disease (see Cohen *et al.*, 2001 for more details on all results discussed here). BSE is extremely unlikely to become established in the US. For example, the (illegal) importation of 10 cows infected with the disease, a rather unlikely event, would on average result in only 3 new cases of BSE in the US and the disease is virtually certain to be gone from the country within 20 years. These results assume the BSE protections in the US would remain unchanged for the 20 years following an introduction. The new cases of BSE would come primarily from leaks in the system protecting animal feed. Importation of one sick animal yields less than one new BSE case in 20 years on average and the disease is quickly cleared from the US system. Similarly, the system shows no potential for an epidemic of BSE from scrapie, chronic wasting disease, or other cross-species transmission of similar diseases. Given our current knowledge, these hypothetical sources of BSE could give rise to one or two cases per year although evaluation of the scrapie hypothesis in the absence of a feed ban cast doubt on this source of disease. Similarly, if the disease does indeed occur spontaneously in cattle, as some have suggested, this would give rise to one or two cases per year with little spread.

Only a very small amount of potentially dangerous tissues would reach the human food supply and be available for possible human consumption following importation of 10 sick cows. We use units of cattle oral ID<sub>50s</sub> to track infectivity from infected animals for potential human exposure. A cattle oral ID<sub>50</sub> is an amount of infectious tissue that would, on average, cause 50% of exposed cattle to develop BSE. The relationship between cattle oral ID<sub>50s</sub> and likelihood of human disease is unknown but European authorities suggest that the cattle disease may be 10 to 100,000 times less virulent for humans (European Union Scientific Steering Committee, 2000). In the entire 20 year period following the importation of 10 BSE infected cows the mean estimate of cattle oral ID<sub>50s</sub> potentially available for human exposure is 35. The concern would be greatest for consumption of cattle brain, spinal cord, and meat derived from advanced meat recovery systems. Some potential exposure would include the presence of spinal cord in certain bone-in cuts of

beef, like T-bone steaks and consumption of cattle intestines. Spontaneous disease or cross species transmission of scrapie are predicted to result in less than 100 cattle oral ID<sub>50s</sub> potentially available for human exposure over 20 years.

Even in an extreme case, the 95<sup>th</sup> percentile, importation of 10 animals leads to only 11 new cases of BSE over twenty years and about 5 times more infectivity potentially available for human consumption. These predictions can be compared with the experience in the United Kingdom with estimates of nearly 1,000,000 infected animals and likely millions of cattle oral ID<sub>50s</sub> available for potential human exposure.

The US measures most effective in reducing the spread of BSE include the ban on importation of beef or beef by-products from the UK (since 1989) and all of Europe (since 1997) by USDA/APHIS, and the feed ban instituted by the Food and Drug Administration in 1997 to prevent recycling of potentially infectious cattle tissues. This mammalian feed ban, with a few exceptions, greatly reduces the chance of BSE spreading from a sick animal back to other cattle through feed. Our model reflects incomplete compliance with the FDA feed ban and we evaluate the potential risks of exceptions to the ban. Measures instituted in meat packing plants by the industry and USDA/FSIS have reduced the opportunity for infectious tissues to contaminate human food.

Specific pathways or practices that have the greatest influence on the possible spread of introduced BSE to cattle relate to compliance with the FDA feed ban and include misfeeding on the farm and labeling of feed and feed products prohibited for consumption by cattle. In addition, the disposition of animals that die on the farm would have significant influence if BSE were introduced to the US. Factors that influence potential human exposure include handling of brain and spinal cord in processing plants and how well inspectors would detect animals with BSE at slaughter.

Our model is not amenable to formal validation since there are no known controlled experiments in which the introduction and consequences of BSE introduction to a country has been monitored and measured. However, as a test of the plausibility of our model, we attempted to model the very small BSE outbreak seen in Switzerland following the introduction of BSE infectivity from the UK. Working with experts in Switzerland, we identified values for specific model parameters necessary to reflect practices and procedures in that country. We then simulated the results of the introduction of BSE infectivity in contaminated feed. Our simulation included risk management actions like feed bans instituted by the Swiss. The model predictions were reasonably close to the Swiss experience, predicting an average of 166 animals with detectable BSE while the Swiss have detected 324 to date. The model also captured the change in BSE cases over time seen in Switzerland (see Cohen *et al.* for further discussion of the evaluation of the Swiss scenario). The ability to replicate reasonably the magnitude and time course of the Swiss outbreak gives some confidence in the structure of our model.

We also evaluated the potential for BSE to have entered the US prior to the 1989 ban on importation of UK cattle and the implications if it had. BSE has not been detected in the

US despite 12 years of active surveillance of high-risk animals. Yet several groups, including the European Union in their Geographically Based Risk Assessment of the US have pointed to the 334 animals brought into the US from the UK between 1980 and 1989. These animals were imported as breeding stock, not as beef or dairy production animals. This may have reduced their potential for exposure to BSE while in the UK. In addition, none came from a farm that saw a case of BSE in animals from the same birth cohort (same birth farm and year). Many came in before BSE was even a recognized disease. The USDA has identified and traced the disposition of all but 173 of these animals.

Using data on the year of birth and importation, the last known sighting of the animals, and the time course of the disease, we have estimated the theoretical amount of BSE infectivity that could have been introduced to the US by these 173 animals. We have then used this in our model to estimate the possible consequences if exposure did indeed occur.

Our analysis finds a better than 80% chance that no BSE came to the US with these animals. We can also rule out the very high estimates of imported disease since they would lead to levels of BSE in America that surely would have been found. In between is a 10 to 14% chance of a small amount of BSE infectivity entering the cattle feed system. If this BSE was introduced it would have spread through the cattle population in the years before the feed ban was introduced. This could have resulted anywhere from zero to several thousand cases of BSE over a 30-year period. The variety of measures taken by the government and industry over the last five years will have arrested the disease and begun its decline to eradication if it was introduced.

There are a number of model assumptions that cannot be verified with confidence, some of which influence the conclusions drawn. With regard to estimating the spread of BSE among cattle, the most influential sources of uncertainty are related to compliance with the FDA feed ban. Within this category, the most important source of uncertainty is the misfeeding rate on farms. Misfeeding prohibited feed (containing ruminant protein) to cattle on farms that raise both cattle and either pigs or chickens completely compromises the feed ban. This practice is the focus of efforts to understand how animals born after the implementation of feed bans in Europe have become infected with BSE. Uncertainty with respect to compliance rates can be reduced with field work and data collection. A second source of uncertainty associated with the feed ban is the proportion of feed produced that is mislabeled (*i.e.*, the absence of proper labels identifying prohibited feed as not to be administered to ruminants).

Our evaluation of potential risk mitigation actions highlights potential measures to further reduce the already low likelihood of spread of BSE in the cattle herd or potential human exposure if it were to arise. Preventing the rendering of animals that die on the farm, possibly of BSE, removes the a great deal of potential contamination in the animal feed chain and reduces average predicted cases of BSE following introduction of 10 infected cows by 77%. Implementation of a UK-style ban on specified risk material (e.g., spinal cords, brains, vertebral columns) from human or animal food reduces the predicted animal cases by 80% and the number of cattle oral ID<sub>50s</sub> potentially available for human

exposure by 95%. These are intended as examples of the evaluation of alternative risk management strategies with the model.

## CONCLUSIONS

In summary, measures taken by the US government and industry have resulted in a robust system to prevent the spread of BSE to animals or humans should it be introduced to this country. Preventing sick animals or contaminated feed from entering the country, ensuring compliance with the FDA feed ban and reducing the potential for infectious tissues to enter the animal or human food supply will ensure the risk remains low. If BSE was introduced to the US, as suggested by some observers, the course of the disease has been arrested and it is destined for eradication by the measures currently in place.

It certainly is impossible to rule out the chance that a case of BSE is identified in the US. There could even be a vCJD case that cannot be traced to exposure elsewhere. However, our analysis suggests that it is very unlikely that BSE will become a major animal or public health problem in America.

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# AMINO ACID DIGESTIBILITY OF ANIMAL PROTEIN MEALS

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Animal protein meals are important feedstuffs in poultry nutrition. These ingredients are high in protein but their amino acid digestibility can vary. There are several factors that have been reported or hypothesized to influence amino acid digestibility of animal protein meals. This paper will summarize some of our research during the last few years to quantitate the effect of factors such as raw material source, processing systems, processing temperatures, processing pressure and ash on the amino acid digestibility of animal meals, with the emphasis being on meat and bone meal (MBM). A new in vitro assay, the immobilized digestive enzyme assay, will also be discussed for predicting in vivo amino acid digestibility of animal protein meals.

## EFFECT OF RAW MATERIAL SOURCE, PROCESSING SYSTEM AND PROCESSING TEMPERATURE

A study was conducted in cooperation with the Fats and Proteins Research Foundation in attempt to identify the major commercial factors affecting protein quality of feather meal (FM), meat and bone meal (MBM), and poultry by-product meal (PBPM). Six samples of FM, 32 samples of MBM, and 12 samples of PBPM were processed in different rendering systems at two different temperatures (low vs high). The raw material source also varied for the MBM (beef, pork, mixed species). Some of the results for the MBM part of the study are summarized in Tables 1 and 2. Although there were some significant differences, raw material source did not have any substantial or consistent effect on amino acid digestibility (Table 1). The type of processing system and processing temperature significantly affected amino acid digestibility (Table 2). The digestibility of Lys and Cys was considerably higher for MBM produced in processing System B than in System A. Moreover, System B generally yielded very high Lys digestibility of 90% or greater. In addition, amino acid digestibility was higher when the MBM was processed at the lower temperature in both Systems A and B, but the temperature effect was greater in System A. At least part of the probable explanation for the lower amino acid digestibilities for System A is that the MBM was processed at higher temperatures than in System B. Cystine was the amino acid that was most affected by processing system and temperature, with the high temperature used in System A yielding very low Cys digestibilities. Finally, it is important to note that the results for System B show MBM with very high amino acid digestibility can be produced with good processing procedures.

Table 1. Effect of Raw Material Source on Amino Acid Digestibility (%) of Meat and Bone Meal<sup>1</sup>

Source	Lys	Cys	Met	Thr
Beef	84 <sup>a</sup>	51 <sup>a</sup>	89 <sup>a</sup>	82 <sup>a</sup>
Pork	84 <sup>a</sup>	46 <sup>a</sup>	88 <sup>a</sup>	78 <sup>b</sup>
Mixed species	80 <sup>b</sup>	45 <sup>a</sup>	86 <sup>b</sup>	77 <sup>b</sup>

<sup>a-b</sup>Means within a column with no common superscript differ significantly ( $P < 0.05$ ).

<sup>1</sup>Wang and Parsons (1998a).

Table 2. Effect of Processing System and Temperature on Amino Acid Digestibility of Meat and Bone Meal<sup>1</sup>

Processing System	Processing Temperature (°C)	Digest. Coefficient (%)	
		Lysine	Cystine
A	132	85	39
A	152	78	20
A	132	81	50
A	152	71	31
B	110	92	71
B	140	90	62
B	110	91	59
B	140	87	51

<sup>1</sup>From Wang and Parsons (1998a).

### EFFECT OF PRESSURE PROCESSING

During the last three years, we have conducted research with MBM to assess the effects of pressure processing on amino acid digestibility. The reason for evaluating pressure processing is due to concerns of bovine spongiform encephalopathy (BSE). The feeding of BSE-infected MBM to ruminants may cause BSE. It is suspected that the consumption of meat from BSE-infected cattle may, in turn, cause Creutzfeldt-Jakob Disease (CJD) in humans. Consequently, extreme restrictions have been placed on the feeding of MBM in the United Kingdom and the feeding of MBM containing ruminant tissue to ruminants has been banned in the U.S. BSE and CJD are caused by heat-stable prion proteins that can be at least partially inactivated by pressure cooking. The European Union requires that MBM be processed at 3 atmospheres (30 gauge psi) for 20 minutes at 133° C (271° F) to reduce the risk of BSE and CJD. It is possible that MBM may have to be pressure processed in the U.S. in the future. However, any future requirements/regulations for MBM processing are unknown. Therefore, several different processing pressures were evaluated in our study.

Processing conventionally-rendered MBM at 15, 30, 45 or 60 psi for 20 min. influenced amino acid digestibility (Table 3). Pressures of 15 and 30 psi produced moderate depressions in

digestibility of most amino acids, including Thr, Lys and Met. The reductions in digestibility of Cys were greater than those for the other amino acids. Increasing the pressure to 60 psi produced large decreases in amino acid digestibility for all amino acids, with by far the greatest reduction occurring for Cys. The large reduction in digestibility at 60 psi was due to both the destruction of amino acids and decreased digestibility of amino acids that were not destroyed.

Table 3. Effect of Pressure Processing on Amino Acid Digestibility Coefficients (%) for Meat and Bone Meal<sup>1</sup>

Gauge pressure (psi) <sup>2</sup>	Thr	Lys	Met	Cys
0	81 <sup>a</sup>	76 <sup>a</sup>	81 <sup>a</sup>	65 <sup>a</sup>
15	76 <sup>ab</sup>	67 <sup>b</sup>	76 <sup>ab</sup>	48 <sup>b</sup>
30	76 <sup>ab</sup>	68 <sup>b</sup>	76 <sup>ab</sup>	50 <sup>b</sup>
45	73 <sup>b</sup>	62 <sup>b</sup>	75 <sup>b</sup>	46 <sup>b</sup>
60	54 <sup>c</sup>	41 <sup>c</sup>	62 <sup>c</sup>	15 <sup>c</sup>

<sup>a-c</sup>Means within a column with no common superscript differ ( $P < .05$ ).

<sup>1</sup>Shirley and Parsons (2000).

<sup>2</sup>Meat and bone meals processed for 20 min. at the specified pressure.

#### EFFECT OF ASH CONTENT

Another variable that is alleged to influence protein quality and amino acid digestibility of animal meals such as MBM and PBPM is ash content. Meals that contain higher ash are generally considered to be of lower protein quality and have lower amino acid digestibility. However, when one reviews the literature, there are limited data to support the effect of ash on protein quality and little or no data to support an effect of ash on amino acid digestibility. The partial results of one of our recent studies to evaluate the effect of ash for MBM are summarized in Table 4. Increased ash content from 9-44% had little or no negative effect on amino acid digestibility. There was a decrease in digestibility of amino acids when the ash increased to 63% (all bone sample). In contrast to amino acid digestibility, increased ash did have a negative effect on protein quality as measured by protein efficiency ratio (PER) in a 10-day chick growth trial. In the PER trial, 10% CP diets were fed in which the MBM provided the only source of dietary CP. The PER values were calculated by dividing the weight gain (g) by the protein intake (g). The reduction in protein quality (PER values) due to increased ash was not due to reduced amino acid digestibility but was due to poorer total amino acid balance or profile; that is, the analyzed level of sulfur amino acids and Trp per unit of CP decreased as the ash content increased. The latter effect is probably due to the increased bone content in the higher ash samples. Bone contains approximately 25 to 30% CP and the protein is of very low quality due to extreme deficiencies of sulfur amino acids, particularly Cys, and Trp. Thus, our initial results indicate that the protein quality of MBM does, indeed, decrease with increasing ash content, but the reduction is due to a poorer balance of total amino acids, not decreased amino acid digestibility. Similar results were obtained for PBPM (Johnson and Parsons, 1997).

Table 4. Amino Acid Digestibility and Protein Efficiency Ratio (PER) of Meat and Bone Meals Varying in Ash<sup>1</sup>

Ash (%)	Lys digest. (%)	Met digest. (%)	PER
9	89 <sup>a</sup>	88 <sup>a</sup>	-
16	86 <sup>a</sup>	87 <sup>a</sup>	3.3 <sup>a</sup>
26	79 <sup>b</sup>	81 <sup>a</sup>	3.0 <sup>b</sup>
35	82 <sup>ab</sup>	82 <sup>a</sup>	2.1 <sup>c</sup>
44	85 <sup>a</sup>	82 <sup>a</sup>	1.7 <sup>d</sup>
63	72 <sup>c</sup>	53 <sup>b</sup>	-

<sup>a-b</sup>Means within a column with no common superscripts are different ( $P < 0.05$ ).

<sup>1</sup>Shirley and Parsons (2001).

### BIOAVAILABILITY VERSUS DIGESTIBILITY OF AMINO ACIDS IN ANIMAL PROTEIN MEALS

A question is sometimes raised concerning the accuracy of digestibility assays and the possibility that they may overestimate actual bioavailability of amino acids for protein synthesis. This question is relevant since digestibility assays measure digestion and absorption of amino acids, but not utilization. It is possible for amino acids to be absorbed in forms that are not suitable for utilization (Batterham, 1992). Therefore, we selected two MBM that varied substantially in digestibility from the raw material source-processing system-temperature study discussed earlier and determined Lys and Met bioavailability by slope-ratio chick growth assay (Wang and Parsons, 1998b). The chick bioavailability values were generally in good agreement with, although sometimes slightly lower than, digestibility values determined in cecectomized rosters (Table 5). The results indicated that most (90% or more) of the digestible Lys and Met in the two MBM was bioavailable for protein synthesis and utilization.

Table 5. Bioavailability Versus Digestibility of Lys and Met in Low and High Quality Meat and Bone Meal<sup>1</sup>

Meat and bone meal	Digestibility (%)		Bioavailability (%)	
	Lys	Met	Lys	Met
Low quality	71	83	64	77
High quality	92	91	93	80
Mean	82	87	78	78

<sup>1</sup>From Wang and Parsons (1998b). Digestibility determined by cecectomized rooster assay. Bioavailability determined by slope-ratio chick growth assay.

### AN ENZYME-BASED AMINO ACID DIGESTIBILITY ASSAY FOR ANIMAL PROTEIN MEALS

There continues to be a great need for rapid laboratory assays that can be used to predict or estimate protein quality of animal meals so that expensive, time consuming, animal trials do not have to be conducted. It has been shown previously that the pepsin N digestibility test is somewhat useful, particularly if the level of pepsin is reduced from .2 to .002 or .0002%. A new

system of immobilized enzymes has been developed by Dr. Chuck Schasteen's group at NOVUS International and the method is known as the Immobilized Digestive Enzyme Assay (IDEA) (Schasteen *et al.*, 2002). The original IDEA system uses pepsin in a low pH digester followed by neutralization and digestion with chymotrypsin, trypsin and intestinal peptidase in a second digester. The original system has been modified using glass derivatization and enzyme immobilization to produce a kit for MBM that will yield results within four hours. The IDEA assay produces amino acid digestibility values that are highly correlated with cecectomized rooster digestibility values determined in my lab (Table 6). Recently, the assay has been extended to include FM and PBPM and is currently being evaluated for fish meal. The assay also works well for soybean meal. Research with the IDEA assay indicates that it is a rapid, robust and inexpensive predictor of amino acid digestibility in several important feedstuffs.

Table 6. Correlation Between the Immobilized Digestive Enzyme Assay (IDEA) and In Vivo Digestibility in Cecectomized Roosters for Meat and Bone Meal<sup>1</sup>

Amino acid	Correlation coefficient (r)	In vivo digestibility range (%) <sup>2</sup>
Lys	.94	43-75
Met	.94	61-79
Cys	.95	14-67
Thr	.96	58-80
Arg	.91	74-85
Val	.89	59-78
Ile	.91	59-80

<sup>1</sup>From Schasteen *et al.* (2002).

<sup>2</sup>Range in cecectomized rooster digestibility among samples used in the correlation with the IDEA values.

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# FAT UTILIZATION AND ENERGY PREDICTION

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## 1. INTRODUCTION

This will be a “hard-hitting” presentation, so it is important to start with a fundamental issue: the problem with fats and oils employed as raw materials in diets for pigs and poultry is that usually they are traded as named commodities; examples are “yellow grease” and “grade 3 tallow” and “choice white grease”– there are many more. It should be obvious to any self-respecting nutritionist that these names are of no value whatsoever when it comes down to the nutritional value of these commodities; it should not be forgotten that nutritional value is the only criterion appearing on the data bases of users (which means, for fats and oils, dietary energy content), although in Europe many will also ask origin (Europe does not allow bovine and ovine fats for obvious reasons).

In a parallel example, how many nutritionists would purchase “soya bean meal” without asking a few fundamental questions, including “what is its protein content”? In Europe, there are a large number of soya bean commodities available, which means that enquiring about protein content is essential (there are other questions asked, but protein content is usually top of the list) . In many countries, those involved in trading fats and oils have little if any knowledge of the value (other than financial) of what they are handling. Even when challenged, they will try and assure clients that what they are offering “is perfectly acceptable and of good nutritional value” without having much nutritional expertise and supporting data.

We are entering a world where reassuring statements from commodity brokers cannot be allowed to continue without supporting evidence. This paper is an attempt to provide scientific data on fats and oils allowing purchasers and users (i.e clients who purchase both fats and oils but also compound feeds containing them) to make informed decisions. The bottom line (which, unusually, is included in the introduction) is that there are ways and means of assessing nutritional value of fats and oils – those who purchase these commodities without due regard to them are running a very big risk.

## 2. UTILISATION OF FATS AND OILS

It is now appropriate to move to nutritional / technological issues, many of which have been known for some considerable time (note the dates of some of the references cited

in this paper). It is well known that digestible fats and oils have approximately twice the dietary energy-yielding potential of digestible carbohydrates (but there is huge variability between the best and the worst fats and oils), they may contain essential fatty acids and fat soluble vitamins (both often overlooked in diet formulation), their physical texture reduces dust in feed mills (there are still a number of explosions within mills attributable to dust) and they promote palatability of diets (optimising feed intake is perhaps the most important aspect of animal production systems whatever the age of non-ruminant livestock). Fats and oils accordingly, have assumed a considerable degree of importance as raw materials in compound pig and poultry feeds in recent years. It is understood that the dietary energy value of any raw material is governed primarily by (a) its chemical composition and (b) the degree to which it is digested by the pig / bird in the supply of energy-yielding substrates. Fats and oils vary considerably in both these factors such that the range of dietary energy values with this group of raw materials is probably greater than for any other. Interestingly, this has not been appreciated widely; there is still a widespread tendency within nutrition texts / tables of feed composition, energy and nutrient requirements to assign a fixed energy value to fats and oils which is completely unjustified.

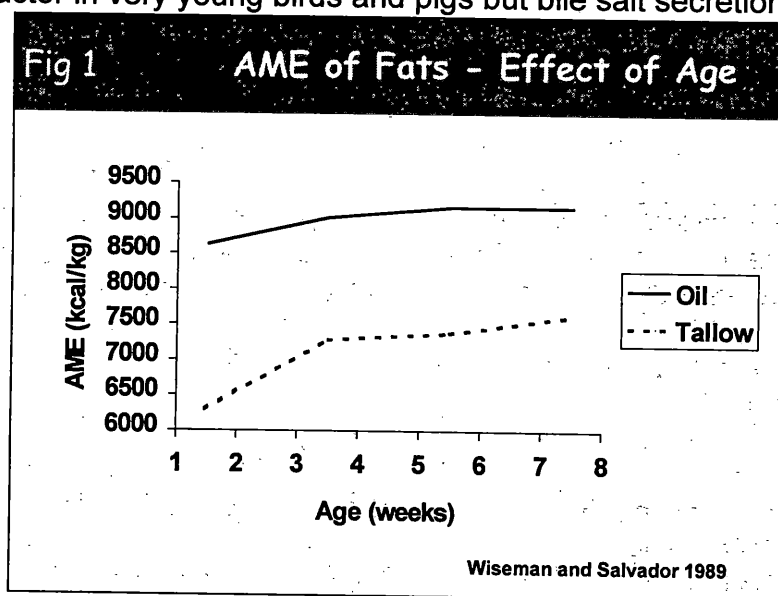
The chemical composition of fats and oils has been examined in considerable detail over the years, but it is this aspect which provides a fundamentally important guide to their nutritional value. It should be noted that, in the nutritional context of this presentation, fats and oils are neutral tri-acylglycerides rather than phospholipids. Furthermore, the chemical variables of greatest importance to energy-yielding potential of fats and oils are degree of saturation and chain length of constituent fatty acids together. As soapstocks and acid oils are also perfectly acceptable components of dietary fats and oils, the proportion of free fatty acids is also of relevance.

There have been numerous reviews on the physiological bases for fat and oil digestion in pigs and poultry (e.g. Freeman, 1976, 1984; Krogdahl 1985). The major site of fat digestion is the duodenum and, basically, this consists of emulsification of dietary fat by conjugated bile salts, followed by hydrolysis of tri-acylglycerides by pancreatic lipase into mixtures consisting essentially of 2-mono-acylglycerides and free fatty acids. The subsequent absorbability of these products is dependent upon their solubility in bile salt micelles. Polar solutes are more readily incorporated into micelles, which explains the relatively higher absorbabilities of unsaturated fatty acids compared to saturated fatty acids and the well-established observation that unsaturated fatty acids have a higher digestibility than those that are saturated (e.g. Renner and Hill, 1961). Accordingly oils, which are relatively unsaturated, have a higher dietary energy value than the more saturated fats - this also explains why hydrogenation of oils (even partial) is associated with a reduction in dietary energy value. The relative superiority of an intact tri-acylglyceride compared to hydrolysed fat in terms of dietary energy value is also well known (e.g. Young 1961; Sklan, 1979), attributable presumably to the importance of mono-acylglycerides in the overall absorptive process.

However, implications for the quantitative assessment of dietary energy value (which in the context of the current paper will be expressed as apparent metabolisable energy for

poultry - AME, and apparent digestible energy for pigs - DE) have only been addressed comparatively recently. There still remains a persistence among applied nutritionists in referring to this class of raw materials in terms of their origin alone (note earlier comments on "brokers") rather than chemical composition. Whilst sourcing of raw materials is assuming greater importance as the food chain seeks to become increasingly proscriptive as to what may or may not be permissible as a raw material in pig and poultry diets, it still remains the case that it is chemical structure and not name or origin that determines AME and DE. The importance of this point is reinforced by the observation that fats and oils are invariably included into diets as mixtures of individual commodities (referred to as 'blends').

Before moving onto a consideration of chemical structure of fats and oils, it should not be forgotten that age of bird / animal is an important variable in utilisation of these raw materials (e.g. with poultry: Fedde *et al.*, 1960; Carew *et al.*, 1972, Wiseman and Salvador, 1989) – see figure 1. It is probable that it is not enzyme lipase itself which is the limiting factor in very young birds and pigs but bile salt secretion.



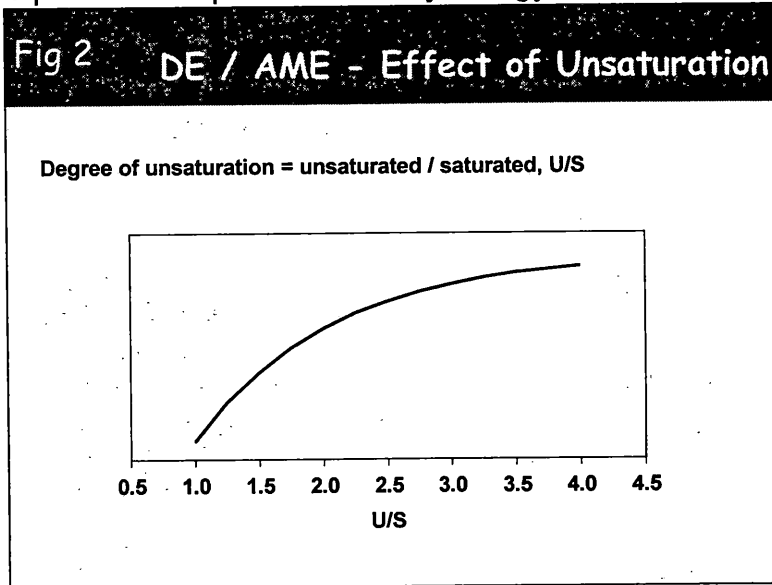
### 3. ROLE OF CHEMICAL COMPOSITION OF FATS AND OILS ON AME AND DE

#### A. Degree of saturation

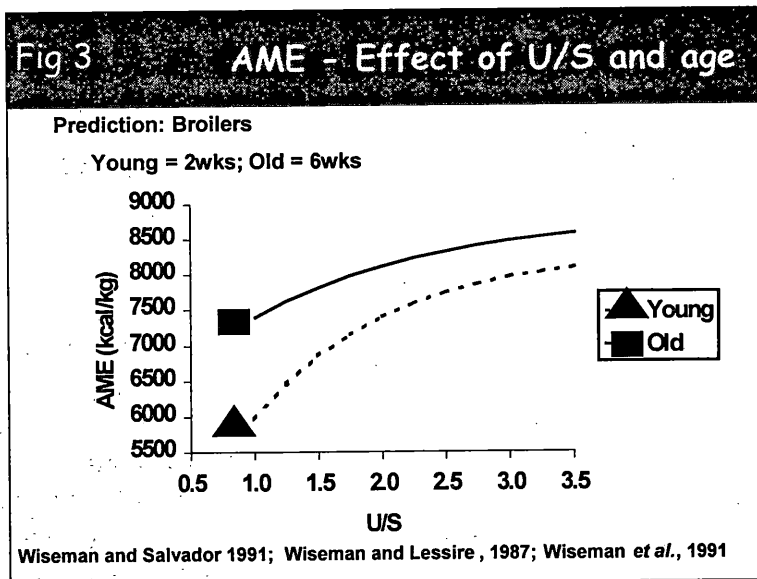
There have been many terms employed to describe degree of saturation of fatty acids. Estimation of the total number of double bonds (through, for example, iodine number) is used widely. However, the value of the term is limited. It would appear that the key difference between fatty acids is the presence (unsaturated) or absence (saturated) of double bonds, not the number of double bonds. Thus an oil based predominantly on oleic acid (C18:1, e.g. rape seed oil) would in all probability have a similar AME and DE value to one based essentially on linoleic acid (C18:2, e.g. soya bean oil) even though the iodine value of the latter would be considerably higher. This explains the lack of precision of equations based on iodine value in attempting to predict the AME and DE of

fat and oil blends.

Accordingly, a more effective approach is to establish the ratio of unsaturated to saturated fatty acids (U/S). From knowledge of the physiology of fat digestion and absorption, the probable response of dietary energy value to U/S is given in figure 2.



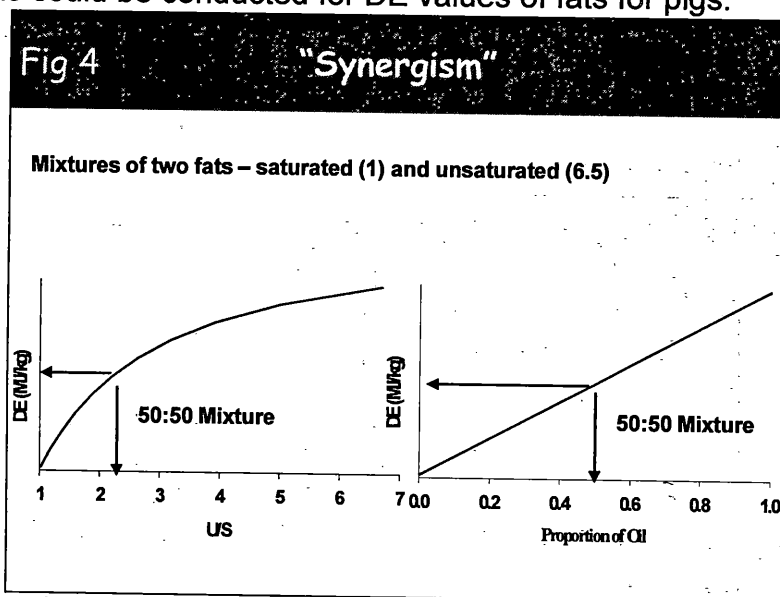
In a comprehensive series of poultry metabolism trials (Wiseman and Lessire, 1987; Wiseman and Salvador, 1991, Wiseman *et al.*, 1991) examining the AME of a number of fats and oils (together with their blends), it was possible to quantify this response (see figure 3); the presence of two responses attributable on age of bird (young referring to 1.5 and old to 6 weeks of age, with the latter extending to adults) is based on the well-known improvement in dietary utilisation of fats the older the bird. Age effects in pigs are less pronounced.



Similar studies have been conducted with pigs and further detailed metabolism trials

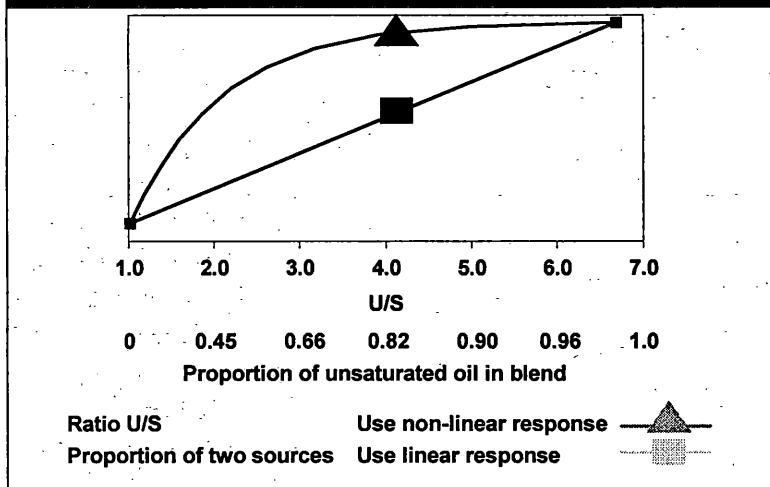
with nursery / growing / finishing animals were conducted by Powles et al (1993a and b). Whilst trends were similar to those found in broilers, the differences in response to changing U/S were less dramatic.

It has been thought for some considerable time that the effects of degree of saturation cannot be considered in isolation of the phenomenon of 'synergism'. This is whereby the AME / DE of a relatively saturated fat is assumed to be improved if blended with a more unsaturated oil. The physiological basis for this in poultry has been established when considering individual fatty acids. Thus the absorbability of stearic acid (C18:0) is improved if blended with linoleic acid (e.g. Renner and Hill, 1961). However, extending this principle to a consideration of fats and oils is not straightforward. Thus the established improvement in the AME with increasing unsaturation (higher U/S) is reliant not only on the greater utilisation of the increasingly important 'U' fraction but also of the 'S' fraction (which, whilst declining in concentration, is still present). Therefore, 'synergism' between individual classes of fatty acid has already been included in the non-linear responses described in figures 2 and 3. What 'synergism' is not is a numerical improvement in the AME of a blend of two sources over and above that which would be predicted from their individual AME values. Thus a 50:50 mixture of two sources with respective AME values of, for example, 8400 and 6400 kcal/kg would yield an AME of 7400 kcal/kg, not higher. The basis for the calculation of AME is presented in figure 4 and 5; the response to U/S is curvilinear whereas the response to the relative proportion of the two sources is linear. It is crucial to appreciate that the U/S of a 50:50 mixture of two sources is not the numerical mean of their individual U/S. Exactly the same analysis could be conducted for DE values of fats for pigs.



The bottom line is that "synergism" when applied to a greater energy value for a mixture of fat and oil commodities than could be predicted from the energy values of the individual components is not valid. "Synergism" in this respect is used frequently by fat brokers (those few who know anything at all about nutrition and those who do not) to sell fat blends based on low grade materials. Nutritionists are well-advised to avoid the "advice" offered by brokers keen to promote the quality of their products through claims which cannot be substantiated scientifically.

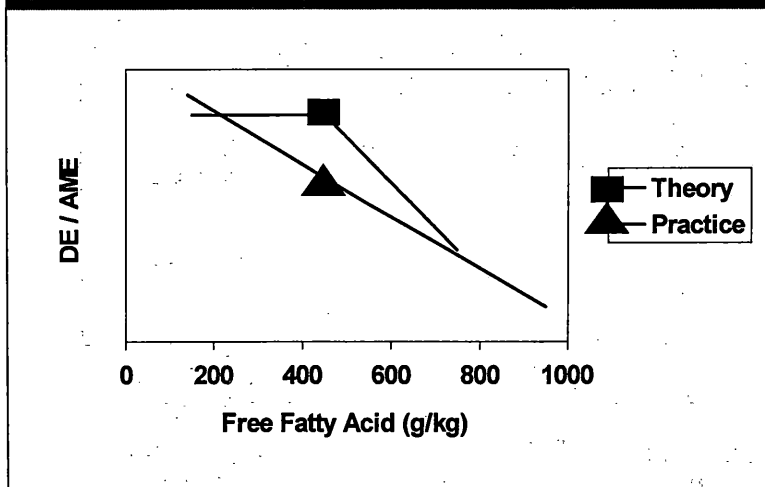
**Fig 5 DE / AME - Effect of Unsaturation**



**B. Inclusion of free fatty acid content**

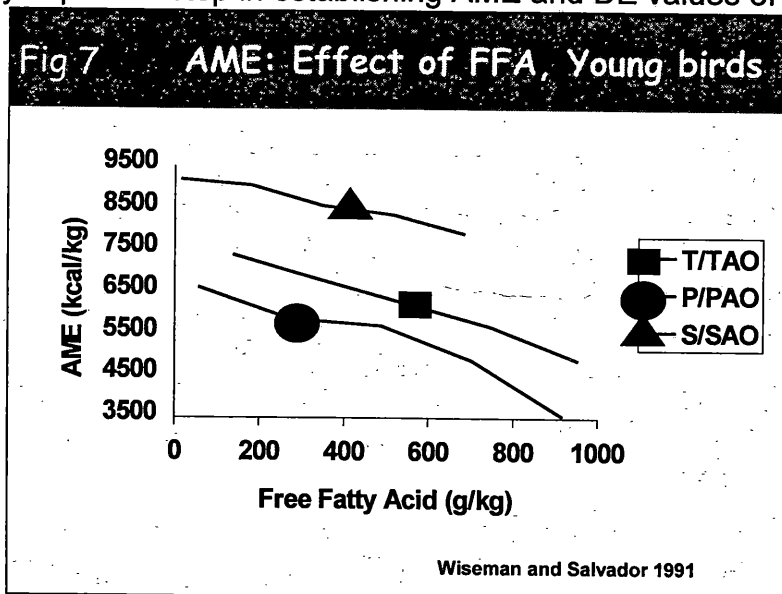
Although degree of saturation of fats and oils is a major chemical variable, it is by no means the only one of relevance to AME and DE values. Whilst hydrolysed fats and oils (i.e. those with high concentrations of free fatty acids - FFA) have lower AME and DE values than their corresponding intact tri-acylglycerides, it is crucial that the response of AME to FFA is assessed to allow the consequences of an incremental increase in FFA to be quantified. Interestingly, the "conventional" wisdom (based usually on the opinion of fat "brokers") is that there is little problem with FFA levels as long as these are below around 450g/kg fat. However, actual biological evaluation reveals that this is not the case (see figure 6 for a comparison of perceived with actual responses)

**Fig 6 DE / AME - Effect of FFA**



The conclusions presented in figure 6 are based on a series of poultry and pig metabolism trials described above (Wiseman and Salvador, 1991, Powles et al 1993a and b); data for poultry are given in figure 7. These trials, in addition to evaluating the

two extreme sources, also determined the AME and DE of mixtures with intermediary FFA content in order to define the response of variable FFA content. It is evident that the reduction in AME and DE with increasing FFA is linear in all cases. This is not to say that FFA levels should be minimised but rather to argue that knowledge of FFA is a fundamentally important step in establishing AME and DE values of fats and oils.



### C. Prediction of energy value

Thus U/S (non-linear response on AME and DE) and FFA (linear response on AME and DE) may now be combined into one predictive model (it having been established that U/S and FFA do not interact) which is presented in table 1.

Table 1. Prediction equations relating the apparent metabolisable energy (AME, MJ/kg - poultry) and digestible energy (DE, MJ/kg - pigs) to ratio of unsaturated to saturated fatty acids (U/S) and free fatty acid content (FFA, g/kg fat) of fats and oils. Age 1 and 2 in poultry refer to 1.5 and 6 weeks of age respectively and to 10-20kg and 35-85kg live weight in pigs respectively.

$$\text{AME or DE (MJ/kg fat)} = [A + B \times \text{FFA} + C \times e^{(D \times \text{U/S})}] / 0.004184$$

#### a. Poultry (AME)

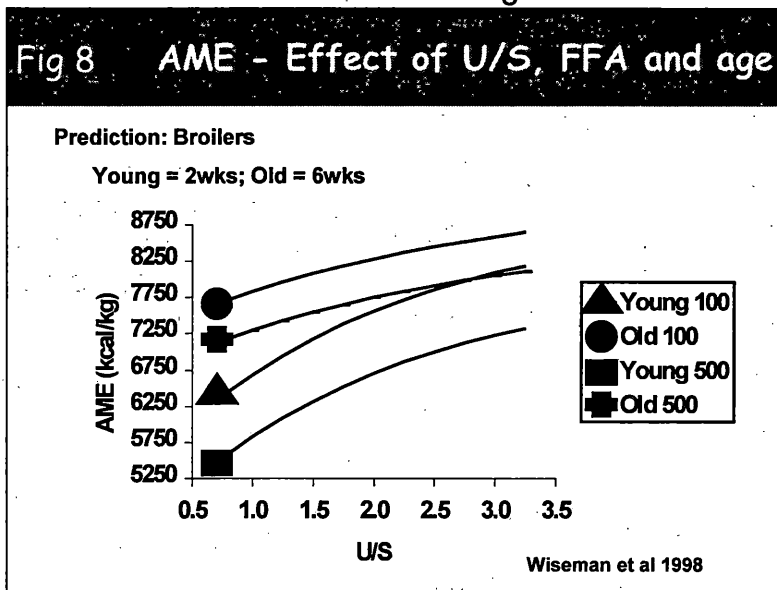
	Age 1		Age 2	
Constant		PV <sup>A</sup>		PV
A	38.112 ± 1.418	0.816	39.050 ± 0.557	0.925
B	- 0.009 ± 0.002		- 0.006 ± 0.001	
C	-15.337 ± 2.636		- 8.505 ± 0.746	
D	- 0.509 ± 0.186		- 0.403 ± 0.088	

b. Pigs (DE)

	Age 1		Age 2	
	Constant	PV	Constant	PV
A	36.898 ± 0.501	0.802	37.890 ± 1.690	0.768
B	- 0.005 ± 0.001		- 0.005 ± 0.002	
C	- 7.330 ± 2.700		- 8.200 ± 1.750	
D	- 0.906 ± 0.452		- 0.515 ± 0.376	

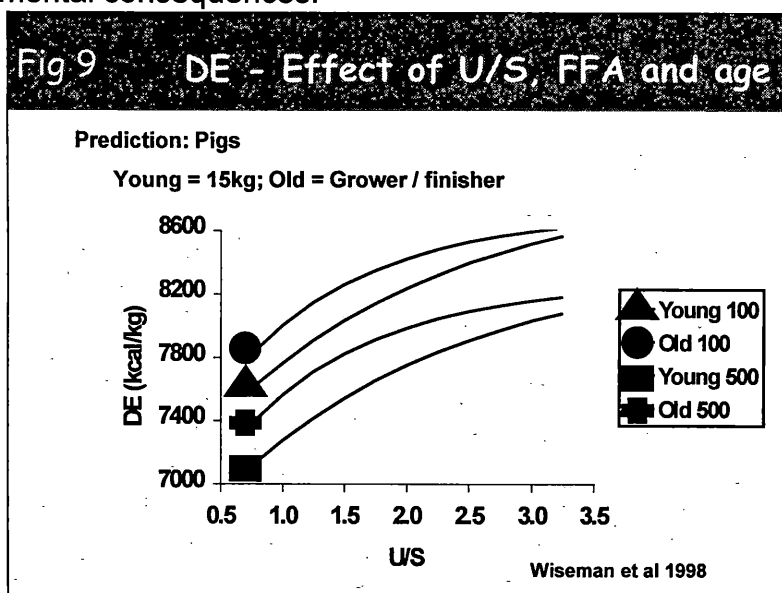
<sup>A</sup> Proportion in variance of dependent variables accounted for by function.  
 To convert MJ to kcal: MJ/0.004184; thus 35 MJ = 8365 kcal

Solutions for these functions over varying U/S, at 2 FFA concentrations and for 2 ages are presented in figure 8 and 9. The range in values is of the order of 3000kcal/kg for poultry and somewhat less at 1500kcal/kg for pigs. It is evident that failure to account for differences in dietary energy value of fats for poultry and pigs would have consequences for bird and animal performance. It has however been pointed out that a difference in AME of 1200kcal/kg between two fat blends would only make a difference of 60kcal/kg to overall diet AME if included at 50g/kg. This would have only modest consequences for performance. However, the fat with the lower energy value should be offered at a price of some 18% below that with the higher.



There is another issue to be considered when handling fats and oils with variable dietary energy values. If a fat blend has a coefficient of digestibility of say 0.9 then, for every 10g of the blend consumed by a broiler, 1g will be excreted; with a coefficient of 0.7, the corresponding output will be 3g. Problems of greasy and capped litter, with their consequent link to carcass quality, may well be associated with high concentrations of

fat in excreta. Generally, increasing levels of fat in bird and animal slurry would also have environmental consequences.



#### D. Chain length

Table 2. Mean chemical analyses of coconut/palm kernel oil blend (CP) and its acid oil (CPAO) - all data expressed as g/kg oil

Fatty Acid Profile <sup>a</sup>	CP	CPAO
8:0	95	42
10:0	71	41
12:0	494	462
14:0	171	170
16:0	78	101
18:0	24	26
16:1	0	0
18:1	53	129
18:2	12	21
18:3	0	0
20+	1	7
U/S <sup>b</sup>	0.07 <sup>c</sup> / 2.66 <sup>d</sup> / 8.78 <sup>e</sup>	0.19 <sup>c</sup> / 2.36 <sup>d</sup> / 6.86 <sup>e</sup>
Free fatty acids (g/kg oil)	13.8	839.0

<sup>a</sup> Notation indicates length of carbon chain followed by number of double bonds

<sup>b</sup> Ratio of unsaturated to saturated fatty acids

<sup>c</sup> All saturated fatty acids included in 'saturated' fraction

<sup>d</sup> Only C14:0, C16:0 and C18:0 appearing in 'saturated' fraction

<sup>e</sup> Only C16:0 and C18:0 appearing in 'saturated' fraction

The majority of fats and oils employed in the manufacture of blends for compound poultry diets consist of fatty acids with chain lengths ranging over the comparatively modest C16 to C20. Those with chain lengths beyond C20 are unlikely to be of major importance as the source of these is invariably fish oils which, because of their oxidative instability, are associated with off-odours. However it is not impossible that fatty acids with chain lengths below C16 (or, rather, sources containing such fatty acids) might be used. Two sources which have high concentrations of short chain fatty acids are coconut and palm kernel oil (not to be confused with palm oil, which is based on C16:0 palmitic acid) and a mixture of the two together with the respective 'acid oil' of high FFA content (table 2) and blends of intermediary FFA content were evaluated by Wiseman and Blanch (1994).

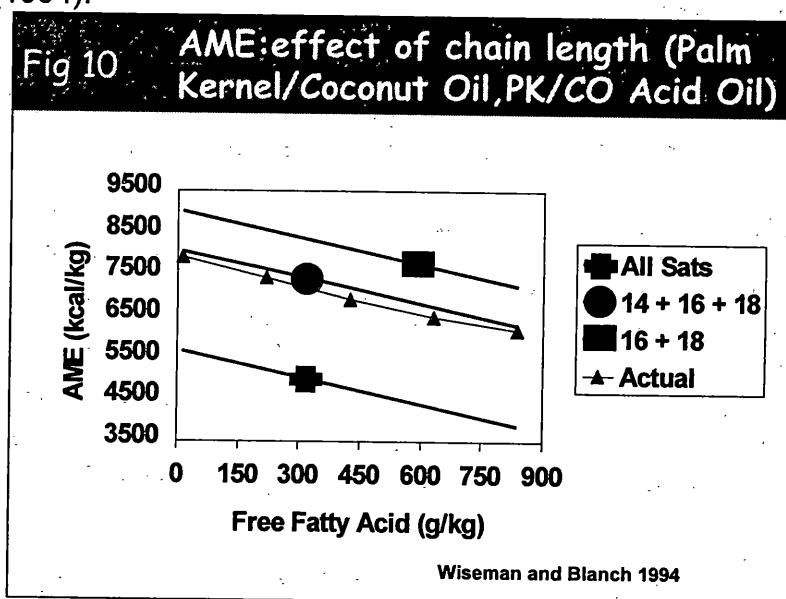


Figure 10 presents data derived from the trial and also the predicted responses as calculated from the model presented in table 1 with 3 means of determination of U/S. It is clear from the responses that, in the calculation of U/S, the 'S' fraction should be based on stearic (C18:0), palmitic (C16:0) and myristic (C14:0) acids but that lauric acid (C12:0) should appear in the 'U' fraction.

#### 4. CHANGES OCCURRING DURING PROCESSING

Fats and oils employed in animal diets are invariably by-products of another process and accordingly have been subjected probably to a range of processing conditions during manufacture and subsequent use such as refining, cracking, rendering and heating (e.g. recovered vegetable oils) which may be responsible for further changes to chemical structure (e.g. Artman, 1969; Wiseman, 1986). As fats and oils are generally relatively unstable (the more so the greater the degree of unsaturation), they are therefore prone to some form of degradation under a variety of conditions. In particular, the presence of oxygen, water and some minerals during heating will be associated with considerable risks of oxidation / polymerisation. A large number of modifications to the

chemical structure of fats and oils following heating have been identified ranging from simple oxidation products through to dimerization and polymerization (linear and cyclic) of both fatty acids and tri-acylglycerides depending upon the substrate in question and the conditions operating. This has prompted numerous studies on the chemical commodities produced during heating and the nutritional implications.

The biological effects of feeding these modified structures are also extremely varied both in terms of the actual response in the animal and its severity. It should be noted that even minor adverse biological consequences would have serious repercussions for output from poultry and pig production. Initially, digestibility and hence dietary energy value will be reduced. The consequences will be that animals will not perform to expectations but also that an increasing amount of dietary fat will pass through the gastro-intestinal tract and be excreted. It is also possible that the presence of modified fat structures within the gastro-intestinal tract may interfere with the overall digestive process such that general nutrient uptake is impaired. Furthermore an actively oxidising fat or oil will destroy other nutrients present, including some vitamins.

Perhaps more concern has been expressed over whether any toxic products are generated following fat and oil heating / oxidation. It does appear however that the majority of these products are only sparingly absorbed and would thus not present a threat. However, in the case of oxidised fats and oils, defence mechanisms in the gut mucosae to prevent absorption may be stretched such that overall nutrient absorption might be reduced. It is certainly the case that death in laboratory animals fed heated fats and oils has been recorded (Andrews *et al.*, 1960) but these are extreme cases related in all probability to physical properties of the materials evaluated rather than their chemical toxicity.

Because of the potential adverse effects of feeding heat damaged / oxidised fats and oils, there has been considerable interest in developing chemical methods for the detection of such damage. It is important to note that any method adopted has to be one that measures all products collectively if it is to have any practical application. Peroxide value (PV) has been employed widely for this purpose, but it is an unsound method. In tracing the change in PV over time (Poling *et al.*, 1962) an increase followed by a reduction was observed. Thus a low PV value may indicate on the one hand a commodity that had not undergone any degradation but, on the other hand, one that had been seriously denatured. PV measured over time might be an improvement, but the rate of production of peroxides may equal their subsequent degradation such that, overall, PV remains constant although the material is deteriorating. Measurement of 'oxidised fat' has been employed but the evaluation is solvent dependent and would not measure those complexes which would not be soluble in polar solvents.

Free fatty acid content has been employed frequently to assess damage to oils used in the human food industry but is inappropriate for fats and oils for incorporation into compound diets. This is because materials of high FFA content are perfectly acceptable ingredients for blends (whilst being of lower energy value than the original tri-acylglyceride). This also explains why assessments of molecular weights or sizes are

inappropriate. Thus a fatty acid trimer (of no dietary energy-yielding value) would generate similar data to a triglyceride (of high value).

A technique which has found favour is one based upon estimating the total non-elutable material (NEM) of a fat or oil through quantitative gas-liquid chromatography (Walkling *et al.*, 1975, Edmunds, 1990) incorporating a glycerol correction (glycerol, the backbone of tri-acylglycerides, is present following hydrolysis and derivatisation of fatty acids; as such it would appear in the NEM fraction although it is not associated with 'damaged' structures). Whilst this method merely measures collectively most degraded structures within fat or oil, it does at least provide guidance as to whether the commodity (for example some recovered vegetable oils from frying operations) may have been excessively heated.

The possible reduction in dietary energy value likely to result from damage was studied by Wiseman *et al.* (1992) employing a refined sunflower oil which was, subsequently, extensively heat damaged (table 3).

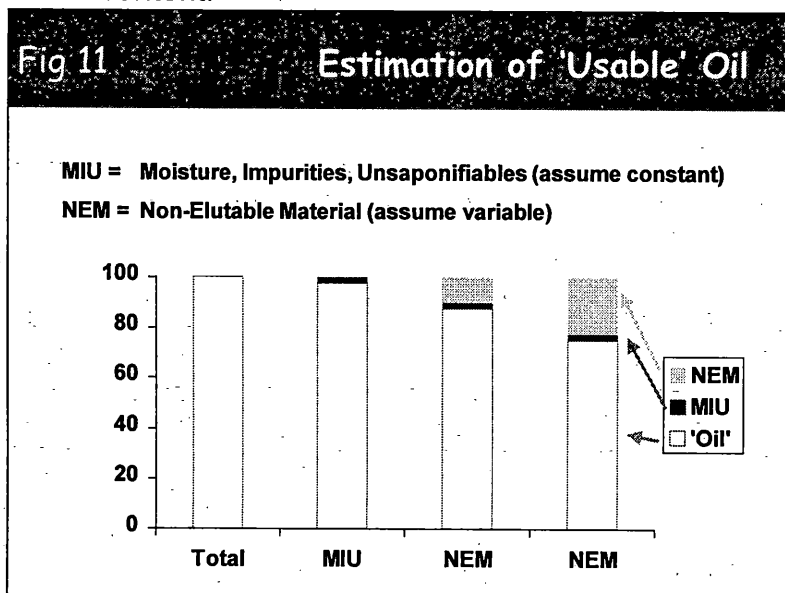
Table 3. Mean chemical analyses of refined (RS) and sunflower acid oil (SAO) - all data expressed as g/kg oil

Fatty Acid Profile	RS	SAO
14:0	0.7	1.4
16:0	69.3	82.4
17:0	2.0	2.7
18:0	44.7	47.9
16:1	1.1	2.0
18:1	196.1	190.5
18:2	651.1	619.6
18:3	1.3	2.0
20+	16.4	25.3
Other	17.3	26.2
U/S	7.32	6.25
Non-elutable Material	41.0	136.6
Free fatty acids	0.0	388.0
Water	2.0	8.2
Unsaponifiable material	0.6	82.0
Oxidised fatty acids	0.4	14.8
Impurities	0.01	0.1

Chemical analysis revealed an increase in FFA and NEM content following such treatment but little difference in the proportion of individual fatty acids. The two commodities (together with mixtures of the two) were evaluated for AME and data generated were compared with values predicted from U/S and FFA. It was evident that the dietary energy value of the NEM fraction, in this material, was of the order of zero.

This indicates the problems identified with heat damaged fats, although no account was taken of associated issues of the presence of the NEM fraction (e.g. reduction in general nutrient uptake).

The consequences of failure to record NEM are presented in figure 11. The conventional means of quality control of fats and oils (other than U/S and FFA) are based on 'MIU'. However, in the example given, two sources with identical MIU and assumed equal FFA and U/S, would not be of the same AME because source B would have a higher NEM content.



It should not be forgotten that there are very much more serious consequences of heating fats and oils if they are contaminated with organochlorines and other similar pesticides which, on heating, will generate the extremely toxic dioxins. In addition, because many industrial waste materials are fat soluble, they may enter the fat and oil market. The most recent very serious consequence of this in Europe was in Belgium where feed grade fats were found to be contaminated, resulting in almost total collapse of the livestock sector as products destined for human consumption were removed from sale. Quality control of fats and oils is therefore not limited to their energy value, but should include more comprehensive tests designed to confirm product safety. Because of the greater risks of contamination in recovered vegetable oils, this commodity as a whole is not regarded favourably.

#### 4. CONCLUSIONS

There is unfortunately a considerable amount of "myth and magic" in respect of fats and oils. Fortunately there has been considerable effort expended on structured programmes designed to quantify nutritional value of these very valuable raw materials in diets for non-ruminants. Increased confidence in the use of fats and oils is a positive outcome of this work and the industry should now be in a better position to utilise them effectively. Comprehensive quality control is the key to establishing confidence in the commodities.

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# **REALIZING THE VALUE OF ANIMAL PROTEINS IN COMMERCIAL FORMULATIONS**

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This paper was not available at press time.

# PHOSPHORUS AND ANIMAL MANURES FROM A SOIL SCIENTIST'S PERSPECTIVE

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## INTRODUCTION

Phosphorus (P) is an economically important input in both crop and livestock production systems (Mallarino and Blackmer, 1992; Valk et al., 2000). The P compounds commonly found in soils are mostly unavailable for plant uptake because of low solubility and slow mineralization rates of organic compounds. Application of P in fertilizers or livestock manure is a critical part of modern crop production systems. On the other hand, agriculture has been identified as an important source of water quality degradation from excessive P. Water quality problems associated with excess P include accelerated eutrophication, low oxygen levels, reduced aquatic species diversity, turbidity, and undesirable taste and odor in municipal water supplies (Carpenter et al., 1998; Sharpley et al., 1994). A challenge in agriculture is to manage P inputs to achieve economical production while minimizing losses to surface water. For the livestock producer, this challenge must be met through comprehensive management of diet, manure handling and application, and cropping systems. This paper focuses on soil P and water quality issues that relate to the management of P in manure.

## SOIL PHOSPHORUS

Soil P is made up of a wide range of organic and inorganic compounds that vary in biochemical reactivity. Three simplified pools of soil P are soluble P, reactive P, and stable P (Fig. 1). Soluble P in the soil is plant available and is predominantly made up of orthophosphate anions (i.e.:  $\text{H}_2\text{PO}_4^-$ ,  $\text{HPO}_4^{2-}$ ). Soluble P makes up only a very small portion of the total P in soil, often less than 1% (Brady and Weil, 1999). Reactive P is found in fresh organic material or in moderately soluble minerals. The stable P pool makes up the largest portion of soil P and consists of crystalline compounds with very low solubility and stable organic compounds that are not plant available. From a crop production standpoint, soluble P is the most critical pool. Plants take up P only in the orthophosphate forms. When P is taken up from the soluble P pool, it can be replaced from the reactive P pool.

Soil testing for P is an effective way to assess the P supplying capacity of the soil and need for P application. The most common laboratory methods used to quantify soil phosphorus are collectively known as soil test phosphorus (STP) methods. The STP determination estimates the concentration of P that is plant available using a chemical extraction. The P extracted included all of the soluble P and a portion of the reactive P pools (Fig. 1). The soil test is used to categorize the soil P level as low, medium, high, and very high (or excessive). These STP categories are based on long term research data

and correspond to the probability of a crop yield increase from applied P (Figure 2). Soils in the very high STP category have a low probability of a crop response to added P.

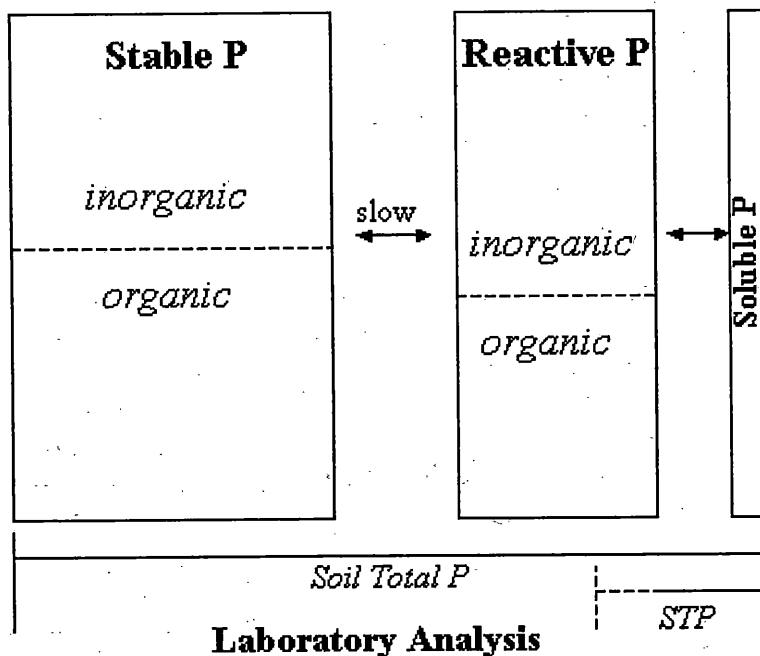


Figure 1. An illustration of three pools of soil phosphorus. The relative size of each pool varies with soil type. Soluble P is always the smallest pool, usually about 1% of the total P.

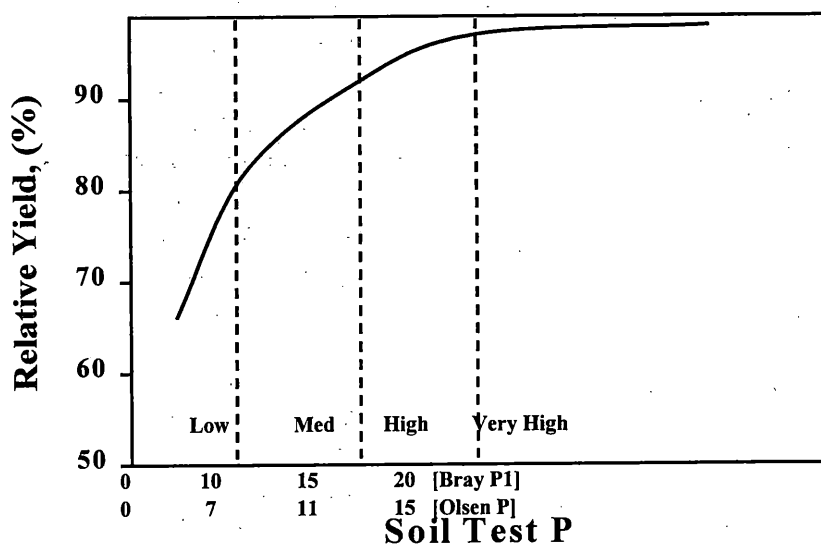


Figure 2. Soil test P categories and their effect on relative crop yield.

## SOIL REACTION TO APPLIED P

When fertilizer P or manure is applied to the soil, the concentration of plant available P increases. Over time, however, a large fraction of added P will be taken up by crops, react with other soil constituents to form insoluble minerals, or be sorbed onto mineral or organic surfaces. Because of these P fixing processes, the addition of fertilizer or manure P does not result in an equal (1:1) increase in STP. For calcareous soils in Minnesota, annual applications of 100 lb P<sub>2</sub>O<sub>5</sub>/ac to corn and soybeans corresponded to STP increases from 2.0 to 2.5 ppm/yr (Randall et al., 1997).

Many years of P fertilization at rates exceeding the amount of P removed by crops have resulted in elevated STP levels in many agricultural soils (Sharpley et al., 1994; Sims, 1992). In 1998, the average STP for Minnesota soils analyzed at the University of Minnesota Research Analytical Laboratory was 30 ppm (Bray P1; Bray and Kurtz, 1945), corresponding to the very high STP category. This average is made up of a wide range of soils, including some that are natively high in P, many that are low testing in P, and some soils with excessively high P concentrations.

Soils with the highest STP levels are generally found in fields where repeated manure applications have been made (King et al., 1990). A recent sampling of soils from livestock farms in Minnesota identified sites with STP concentration as high as 275 ppm (Bray P1). A question that is often asked is how long it will take to reduce high STP concentrations to threshold levels. For soils very high in P, it takes many years to reduce STP to levels where crops would respond to P application because stable P is slowly released to more available forms (Sharpley and Rekolainen, 1997). A clay loam soil was cropped in a corn-soybean (*Glycine Max* L.) rotation with no P applied for 8 years in order to observe STP decline rates. The STP decline rate (Fig. 3) was 1.6 ppm yr<sup>-1</sup> when the initial STP level (Bray P1) was 20 ppm and was 2.9 ppm yr<sup>-1</sup> when the initial STP level was 40 ppm (Randall et al., 1997). Thus, for this soil it would take approximately 10 years of cropping to reduce the STP from 40 mg kg<sup>-1</sup> (ppm) to 15 mg kg<sup>-1</sup> (ppm). Much longer times would be required for soils with STP in the hundreds.

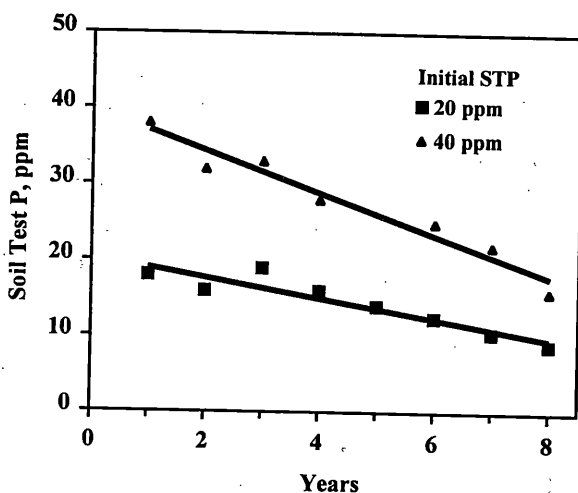


Figure 3. Decline rates of STP for an Aastad clay loam soil in Minnesota with differing initial STP concentration over an 8-yr period with no fertilizer P added. The site was managed in a corn-soybean rotation (modified from Randall et al., 1997).

### THE PHOSPHORUS INDEX

When STP exceeds levels where a crop response to P application is expected, the risk for P loss in runoff water becomes a more critical consideration. However, STP alone is not a good means of assessing environmental risk because it does not assess the probability that P will move into a sensitive water body. For example, a soil classified as 'very high' in STP indicates a low probability of a crop response to P addition, but it does not indicate that water quality risk is high. The risk of high soil test P on water quality depends on transport factors such as erosion and runoff potential and is site-specific. Another problem with the use of STP alone for water quality risk assessment is that STP does not measure all of the forms of soil P that may cause eutrophication. The P Index is a risk assessment concept that has been developed widely in the U.S. since about 1995. The value of P indexing is that source and transport factors are considered simultaneously to evaluate risk and help make management decisions that address environmental and production issues. A closer look at P Indexing will provide insight into the interactions of soil, manure, and water quality.

Phosphorus indices assess the risk of off-site P movement from fields or watersheds (Birr and Mulla, 2001; Gburek et al., 2000, Lemunyon and Gilbert, 1993). Although several different versions of P indices have been developed, all indices have a common set of factors used to evaluate the environmental risk of a site. These core factors can be divided into P source factors and transport factors.

*Source Factors.* The most important P sources used in assessing water quality risk are soil P and P applied as fertilizer or manure. The most significant risk associated with

soil P occurs when erosion transports soil particles into streams, rivers, and lakes. When this occurs, P from all three soil pools (Fig. 1) is mobilized. In the Minnesota P index, the STP concentration is used to assess the soil P source, but STP is converted to soil total P concentration using a calibration equation (Fig. 4). The intercept on this figure illustrates that soils with a very low STP still have a significant concentrations total P. This means that for some locations, high erosion potential and low STP may create a larger water quality risk than a site with low erosion but high STP.

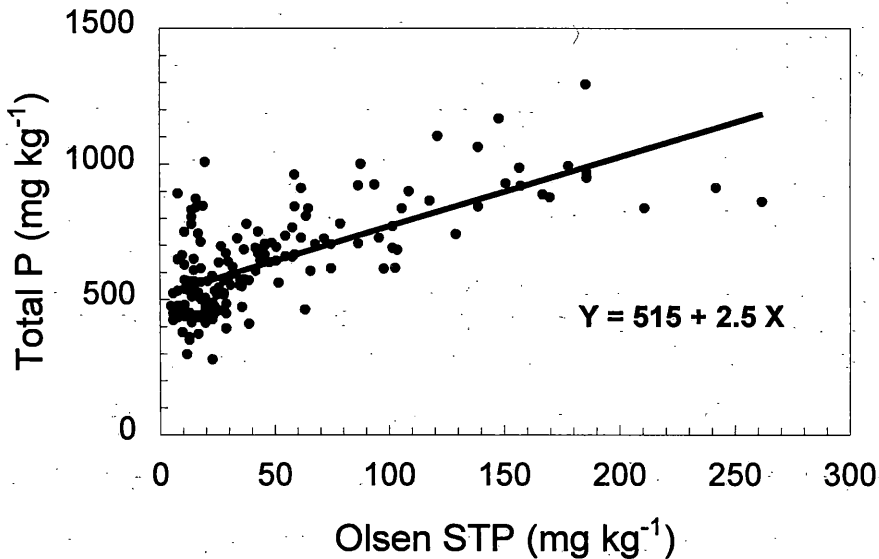


Figure 4. Relationship between Olsen soil test P (STP) concentration and Total P concentration for 160 Minnesota soils.

Phosphorus applied in fertilizer and manure are additional source factors considered in P indices. Applied P affects risk in two ways. First, the applied P increases soil P concentration and the potential for P loss in an erosion event. Secondly, there is a risk that applied P materials can be transported to waters before they have been incorporated and reacted with the soil. The direct losses of applied P materials are most likely when a runoff follows shortly after the P application and the highest risk occurs for surface applied P without incorporation. The risk of direct transmission of P is dramatically reduced when P is incorporated or injected. Phosphorus indices are sensitive to these P management issues and weigh the relative risks of increasing tillage to incorporate applied P in erosion prone areas versus limiting incorporation of applied P in order to maintain cover at the soil surface.

*Transport Factors.* Transport factors in P indices are soil erosion potential, runoff potential, and the proximity of a site to surface waters. In some regions of the U.S., losses of P through leaching and tile drainage can also be significant and P leaching is included as a transport factor (Sims et al., 1998). Soil erosion potential is most often estimated using the Revised Universal Soil Loss Equation. This equation estimates long-

term average annual soil loss and is commonly used by state and federal agencies. Methods for estimating runoff potential vary among P indices. Most all estimates consider soil permeability, slope, and management practices. The importance of proximity to water bodies is primarily because particulate P is subject to deposition during transport from land to water and the probability of deposition increases with distance.

*Computing Site Risk.* Although most P indices are based on similar input variables, the method of computing the site risk can be quite different among versions. In the original P index (Lemunyon and Gilbert, 1993) the overall site risk is calculated by categorizing each source or transport factor as low, medium, high, or very high and associating a numerical score with each category. Further, each source or transport factor is assigned a weight to reflect the relative importance of each factor. The combination of weighting and numerical risk categories creates a simple, unitless risk scale from 0 to >32. More elaborate calculation methods are also used in some P indices (Mallarino et al., 2001).

The Minnesota P index has several unique features specific to the region. In Minnesota, snowmelt runoff is one of the largest hydrologic events of the year and can be a significant transport mechanism for P. In most cases, snowmelt runoff is not highly erosive because of low runoff rates and frozen surface soil. However, soluble P in runoff can originate from crop residues or winter applied manure. Winter application of manure is a common practice in Minnesota and neighboring states. A survey of manure handling practices among Minnesota dairy farmers (Rudstom, 2001) was used to estimate the extent of winter-applied manure. Approximately 11% of all land applied dairy manure in Minnesota is applied during the winter months. The snowmelt component in the Minnesota P index considers the average snowfall in regions around the state and the fall soil roughness condition. Tillage practices with rough surfaces generated across the slope have lower risk of P transport by snowmelt than smooth fields such as no-till.

Another unique component of the Minnesota P index is a reduction in erosion potential for fields with incorporated manure. The reduction in erosion and associated P loss is due to an improvement in soil structure resulting from the organic matter added with the manure (Ginting et al., 1998). A factor in the P Index reduces erosion risk by 25% for fields with incorporated manure. Thus, incorporation of manure concurrently elevates the P source factor, while reducing the transport risk. The importance of incorporated manure relative to other risk factors in the P index is site specific and depends on erosion potential and STP.

Computation of risk in the Minnesota P index is estimated separately for three transport pathways. Transport pathways are erosion, rainfall runoff, and snowmelt runoff (Table 1). Leaching of P to ground water or tile drainage was not considered in the MN P index. For each transport pathway, there are associated P source factors and additional management practice factors.

Table 1. Transport pathways and associated risk factors used to calculate the Minnesota P Index risk score.

<b>Erosion</b>	Average annual erosion is estimated with the Revised Universal Soil Loss Equation. This equation accounts for effects of management practices such as crop rotation and tillage practice. The predicted erosion is converted to the amount of sediment reaching a water body as a function of the distance from field edge to the nearest surface water.
Source Factors	Soil Test P and Applied P. Results from the most recent soil test are adjusted for P additions or crop removal since the last soil test and the adjusted value is converted to total P concentration.
Practices	Reductions in particulate P delivery are accounted for when there are vegetative buffers or structures that promote sedimentation. Structures include sediment ponds, terraces, and impoundments. Erosion rates are reduced for incorporated manure.
<b>Rainfall Runoff</b>	A base runoff map is used to determine the average runoff for a given region in the state. The base runoff is then modified based on the specific soil type and management. Management practices that affect runoff volume include crop rotation and tillage practice.
Source Factors	Soil Test P and Applied P. Results from the most recent soil test are adjusted for P additions or crop removal since the last soil test and the adjusted value is converted to the concentration of dissolved P in runoff. When manure or P fertilizers are surface applied without incorporation, there is an additional source factor that accounts for direct loss to runoff.
Practices	All management practice effects are considered in the transport and source factor calculations.
<b>Snowmelt Runoff</b>	A risk value is assigned by region according to average annual snow depth in March.
Source Factors	Biomass P and Winter Applied P. P in plant residue is based on crop, yield, and tillage practice. The P content of winter-applied manure is a required input.
Practices	Fall Soil Condition Factor. A factor based on tillage type and direction and relating to surface roughness.

The overall P site risk score is the sum of the risk values for each of the three pathways. The calculated risk will be categorized as very low, low, medium, high, or very high. When risk is in the very low or low categories, no change in management is recommended. In the medium risk category, changes in management may be necessary in the future and the producer should be aware of what management practices should be employed to prevent risk from getting higher. In the high and very high risk categories, changes in management practices are recommended. Results from the P Index identify

the causes of the high risk and suggest management practices that will be most effective in reducing risk.

Surface application of manure, without incorporation, is identified in the P Index as a high risk management practice. The level of risk from surface applied manure depends on the runoff potential of the site, the manure P content, and the application rate. Winter applied manure is similarly found by the P Index to be a high risk practice. Incorporation or injection of manure is identified as a way to control risk of P loss. Locations near sensitive waters will be found to pose a high risk when erosion rates or STP levels are high. When high STP is the source of a high risk P Index score, the recommended outcome is to limit any additional P application. However, there are multiple ways to lower the P Index. For a given situation the best management options can be evaluated using the Minnesota P Index. Lowering source levels by limiting P applications, improving methods of P application, or adopting practices that reduce the probability of P transport across the landscape are options to reduce risk.

## SUMMARY

Phosphorus is an important nutrient as an input in agriculture and as a pollutant in surface waters. Fertilizer, manure, and other organic P sources are land applied to support adequate plant growth. Phosphorus (P) is the nutrient limiting algae growth in most fresh water systems. When P in runoff is allowed to enter surface waters the resultant algal bloom results in depleted dissolved oxygen levels and the associated degradation in water quality. Phosphorus is also an important plant nutrient. The challenge that people who make land use decisions have is to balance economic and environmental risks.

The Minnesota Phosphorus Index is a management tool for individual fields or landscapes that provides a numerical risk assessment that P from snow melt and rainfall runoff will enter surface waters. It also allows the user to evaluate management options that can reduce the risk. The P Index will be especially useful in making decisions relative to the land application of manure.

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# MEETING PHOSPHORUS REQUIREMENTS OF RUMINANTS IN AN ENVIRONMENTALLY RESPONSIBLE WAY

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## INTRODUCTION

Public scrutiny of the impact of agricultural practices on the environment is growing. The livestock and poultry industries have been targeted for attention because of their visibility, and for real as well as perceived abuses. Large concentrations of animals in relatively small areas create difficult challenges in terms of odor and nutrient management, but problems of nutrient management can plague small as well as large animal operations. One of the fundamental challenges facing the livestock/feed industries is to recycle the flow of feed nutrients, particularly phosphorus (P) and nitrogen (N), from animal operations back to cropland where they can again be used for crop production. Anything short of this is not sustainable, and will ultimately be unacceptable to the broader public.

To achieve effective nutrient recycling, and to minimize environmental damage, application of manure nutrients must be limited to an amount that crops can utilize. Herein lies the rub. Areas with high livestock densities will have to transport manure nutrients over larger distances to avoid over application of nutrients, or alternatively, relocate animals to where cropland is available for manure application. Full crediting of manure nutrients will be essential, and switching to a phosphorus standard is inevitable. Currently most states permit manure application in amounts that supply the crop need for N. Since the P:N ratio in manure is approximately twice that of the P:N ratio needed by crops, applying manure to meet nitrogen needs results in a build-up of soil P levels.

The Environmental Protection Agency (EPA) is expected to update its definition of and requirements for Concentrated Animal Feeding Operations (CAFO) in December, 2002. Currently, CAFO's are defined as those operations having more than 1000 animal units, with an animal unit equaling 1000 lb of live weight. One thousand beef cows would be equivalent to 700 dairy cows, and an operation with these numbers of animals would be considered a CAFO under current definitions. There has been speculation that EPA may lower the number of animals needed to qualify as a CAFO, but that won't be known until December, 2002. What is expected, however, is that all CAFO's will need to have a nutrient management plan, and that CAFO's located within a priority watershed will be required to limit manure application to crop need for P as well as crop need for N. Many livestock operations in the United States are located in priority watersheds. Switching to a P standard will increase the land area required for manure application, doubling the land area in some cases relative to current requirements. The objective of this paper is to illustrate how eliminating excess P in dairy and beef diets can help producers

meet EPA/DNR regulatory requirements, how this can help the environment, and how this can save money.

## REDUCING PHOSPHORUS EXCRETION IN DAIRY MANURE

There has been much confusion about the P requirement of lactating cows. This is reflected in large differences between feeding standards used by different countries in Europe and North America (Tamminga, 1992). Some of the standards differ by as much as three fold in their estimate of P maintenance requirements, and nearly two fold in the requirement for milk production. Likewise, large differences exist in estimates of P availability in the gut. It is noteworthy, however, that the standards differ relatively little in their final recommendations for P feeding, as extreme differences in maintenance and lactation requirements tend to cancel each other. The NRC (2001) presents an excellent summary of P utilization by dairy cows, and does much to clarify what has been a murky area in the past.

The literature on P utilization and P requirements of lactating cows has been surprisingly consistent. It is in the interpretation of published reports where much confusion has arisen. This confusion has led to feeding of unrealistically large amounts of P in dairy diets. Several surveys (Sansinena et al., 1999; Bertrand et al., 1999; Satter, unpublished information) in the United States during 1999 indicated that dairy diets were formulated to contain approximately .45-.50% P (DM basis), an amount that is about 20% in excess of the requirement (NRC, 2001). This over supplementation of P was costing the U.S. dairy industry about \$100 million annually, as well as increasing risk of environmental damage through eutrophication of lakes and streams. Recent evidence is suggesting that dairy producers have started to reduce dietary levels of P, and that average P concentrations in dairy diets have dropped from about .48% to about .44 or .45%. This is good progress, but it still is about 15% in excess of the lactating cow's requirement.

How have we come to this point of excessive P feeding? There are at least three factors which have played a role. Perhaps most significant is the notion that increasing dietary P will improve reproductive performance. Studies in South Africa (Theiler and Green, 1932) demonstrated that supplementing bone meal to beef cows grazing dry season rangeland improved reproductive performance, as well as growth rates and survival rates. A widely cited field study in England (Hignett and Hignett, 1951) involving 802 dairy cows in 39 herds showed improved first service conception rates when P was supplemented to those herds in the study having the lowest dietary P content. In both of these classic studies, dietary P levels were much lower than current NRC (2001) recommendations, and likely provided insufficient P for maximum rumen microbial growth. Durand and Kawashima (1980) suggested the maximum P requirement for ruminal microbes is 4 g P/kg digestible organic matter in the diet. This would be equivalent to less than .30% dietary P. Extremely low dietary P can inhibit microbial growth, leading to reduced protein and energy supply to the host animal. It is well known that energy and protein supply can influence reproductive performance. Modern dairy diets never approach the low dietary P concentrations that can result in impaired microbial growth in the rumen. There is no evidence that feeding P in excess of NRC (2001) requirements will influence reproductive performance. A summary of 13 trials where reproductive performance of dairy cows fed different levels of P was measured indicated no relationship between reproductive performance and dietary P content (Satter and Wu, 2000). A recent study by Lopez et al. (2002) affirms that feeding P in excess of

NRC (2001) recommendations is without effect on reproductive performance in lactating cows. In this study a total of 267 Holstein cows were randomly assigned at calving to a control diet containing .37% P (dry basis) or to a treatment diet containing .57% P. Cows were fitted with a radiotelemetric transmitter (Heatwatch DDx®) and were bred to natural estrous from day 50 to day 100 and to synchronized estrous after day 100. Weekly ultrasonography was performed from day 50 until pregnancy. Weekly blood samples were analyzed for progesterone (P<sub>4</sub>) concentrations. Dietary P had no effect on any of the reproductive measures made, and no effect on milk production or milk composition. Tables 1 and 2 contain a sampling of reproductive measurements from this study.

Table 1. Characteristics of estrous behavior for lactating cows fed diets containing .37% or .57%P (Lopez et al., 2002).

Characteristic <sup>1</sup>	.37%P (n=159)	.57%P (n=174)	P
Duration of estrus, h <sup>2</sup>	8.7 ± 0.5	8.7 ± 0.6	0.99
Total mounts	7.5 ± 0.5	7.8 ± 0.5	0.68
Total mounting time, sec	25.8 ± 1.8	24.5 ± 1.5	0.59
Average duration of standing events, sec	3.4 ± 0.2	3.4 ± 0.2	0.76

<sup>1</sup>Estruses consisting of only one standing event were removed from the analysis.

<sup>2</sup>Number of hours between the first and the last recorded mount of an estrous period.

Another factor contributing to the overfeeding of P to dairy cows has been the absence of lactation trials showing the absolute minimum of P required to support moderate to high levels of milk production. Without knowing the bare minimum of P needed to support milk production, arriving at a reasonable margin of safety in formulating diets becomes problematic. This uncertainty has led to overly large margins of safety and excessive P in the dairy diet. Information is now available to show that moderate to high producing dairy cows (17,000-28,600 lb milk/lactation) are likely to exhibit beginning signs of P deficiency following long term feeding (1-3 lactations) of diets containing about .3% P (Brintrup et al., 1993; Valk and Ebek, 1999; Wu et al., 2000; Wu et al., 2001).

A third factor contributing to overfeeding of P has been aggressive marketing of P supplements. This has probably been less important than the first two factors mentioned.

Figure 1 is a summary of the status of P nutrition of lactating dairy cows producing > 20,000 lb/305 d lactation (modified from Wu et al., 2001). The bare minimum of dietary P consistent with normal or near normal animal performance is .30%. At this dietary concentration, symptoms of P deficiency may begin to occur. At the other extreme of the continuum in figure 1 is what most dairy producers in the United States are actually feeding.

Table 2. Reproductive measurements for lactating cows fed diets containing .37% or .57%P (Lopez et al., 2002).

Observation	0.37%P	0.57%P	P
Days to first P <sub>4</sub> increase <sup>1</sup>	53 ± 3 (n=133)	53 ± 3 (n=133)	0.97
Days to first natural estrus <sup>2</sup>	68 ± 1.1 (n=103)	67 ± 1.2 (n=109)	0.87
Days to first service	89 ± 2.0 (n=127)	90 ± 2.0 (n=131)	0.87
Conception rate at first AI <sup>3</sup> , %	39.4 (50/127)	42.0 (55/131)	0.67
Overall conception rate at 30 d <sup>4</sup> , %	34.3 (99/289)	38.0 (111/292)	0.35
Overall conception rate at 60 d, %	29.1 (84/289)	31.8 (93/292)	0.47
Pregnancies lost (30 to 60 d), %	15.2 (15/99)	16.2 (18/111)	0.83
Pregnancies lost after 60 d, %	6.0 (5/84)	5.4 (5/93)	0.87
Days open	112 ± 3.5 (n=99)	116 ± 3.8 (n=111)	0.45
Services/conception <sup>5</sup>	2.9 (289/99)	2.6 (292/111)	0.35
Double ovulation rate, %	19.9 (47/236)	18.4 (50/272)	0.66
Anovulatory condition <sup>6</sup> , %	29.9 (40/134)	27.1 (36/133)	0.61

<sup>1</sup>First increase in progesterone concentration >1 ng/ml.

<sup>2</sup>First natural estrus detected by the Heatwatch system between 50 and 100 d.

<sup>3</sup>Number of pregnancies detected at 30 d divided by the number of first services

<sup>4</sup>Number of pregnancies detected at 30 d divided by the total number of services

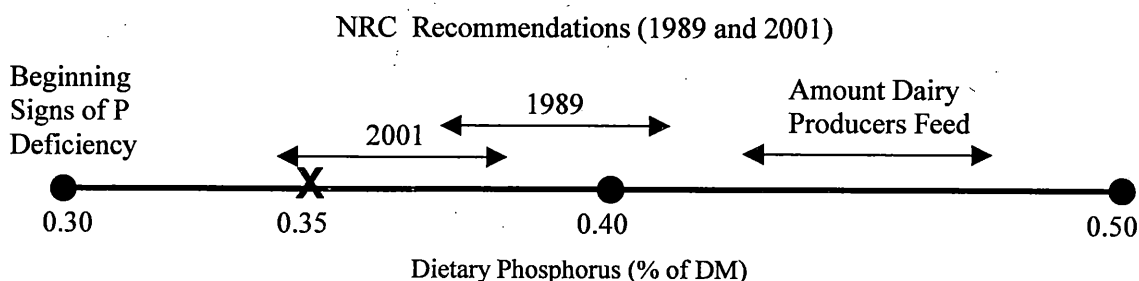
<sup>5</sup>Total number of services divided by the number of pregnancies detected at 30 d.

<sup>6</sup>Cows with no new CL for at least three weekly consecutive ultrasound examinations after d 50.

Figure 1 also shows the requirements for P as indicated by the NRC (1988 and 2001). For ease of illustration, the NRC requirements are expressed in terms of percent P in the diet. This is based on dry matter intakes suggested by the NRC. The most recent NRC (2001) publication has lowered slightly the requirement for P feeding, a change that is fully justified by research results. The NRC presents requirements, and does not include a margin of safety. In calculating the requirement, however, it appears the NRC committee used a somewhat conservative estimate for P availability, or the P absorption coefficient. The NRC (2001) model used P absorption (availability) coefficients of 64 and 70% for forages and concentrates, respectively. We have been examining P availability in some common dairy feedstuffs and with the feedstuffs examined to date, it appears that true digestibility or availability is in the range of 70-85% (Aguerre and Satter, unpublished). The long term lactation studies mentioned earlier would

confirm that the NRC (2001) requirements are more than sufficient, and one might in fact consider the NRC (2001) requirement to include a reasonable margin of safety.

Figure 1. Current status of P nutrition of lactating dairy cows milking > 20,000 lb/305 d of lactation



It is difficult to determine what a reasonable margin of safety is with regard to P feeding. It will depend upon uniformity of milk production of cows within the feeding group, variability of P content of diet ingredients, and how quickly cows exhibit P deficiency symptoms. Variability in DM intake between animals of comparable milk production will also be a factor. The NRC (2001) suggests that Holstein cows weighing 1496 lb, having a body condition score of 3.0, that are 65 mo of age, and producing milk containing 3.5% fat and 3.0% true protein will have a dietary requirement (using a sample diet) of 0.32, 0.35, 0.36 and 0.38% P for milk production amounts of 55, 77, 99 and 120 lb/d. Certainly grouping cows by milk production level would enable a closer match between dietary P and P requirement.

Based on information in NRC (2001) feed composition tables, it appears that the coefficient of variation for P content within a feedstuff listed is about 15%. The NRC (2001) tabular values for P content of feedstuffs appear more accurate relative to the NRC (1988) tabular values, as the older NRC values for P content were systematically lower than recent laboratory analysis (Berger, 1995). This may be a reflection of increased soil P levels in more recent years, since high soil P concentrations can result in elevated plant P content.

Cows lose both calcium and P from bone to help supply these elements in early lactation. Ternouth (1990) suggested that up to 30% of bone P can be removed during early lactation. Based on this estimate for beef cows, a dairy cow weighing 1400 lb could mobilize about 1000g of P in early lactation. Phosphorus mobilized from bone would need to be restored in later lactation, but the sizeable bone reserve provides a buffer against short term P deficiencies that might result from underestimating P content of a batch of feed. Also, mobilized bone P reduces the need for elevated dietary P levels in the first weeks of lactation when feed intake lags behind milk production.

With this background, a reasonable approach might be to formulate group rations using NRC (2001) recommendations to match the average production level of the top 25% of cows in a

feeding group. If this is done, then high production groups in the highest producing herds would have their P requirement met, with a reasonable margin of safety, with diets containing .36-.40% P. This amount of dietary P can be supplied with little or no use of P supplements, and it represents a 15% reduction in P content of the average dairy diet fed in the United States in 2002.

How well does the literature support the NRC (2001) recommendations? Table 3 contains a summary of lactation studies where the control group of cows was fed an amount of P approximately equal to or below the requirement suggested by NRC (2001), and the treatment group received P that was approximately equal to or greater than the NRC recommendation. This series of studies indicate that feeding P in excess of the current NRC (2001) recommendations was without benefit in terms of milk production. The average milk production for the low P groups was 66.9 lb/day, and for the high P groups it was 66.7 lb/day. The NRC (2001) recommendations were of course based on this type of literature information.

Phosphorus fed in excess of the requirement is excreted, with the vast majority appearing in the feces. Typically cows fed just enough P to meet their requirement will excrete < 1 g P/d in urine. Cows fed P 20-30% in excess of their requirement may excrete 3-5g P/d in urine (Wu et al., 2000). Table 4 contains results from a lactation study where cows were fed diets containing .31, .39 or .47% P for a 308 d lactation (Wu et al., 2001). Based on bone P and ash content, cows fed the .31%P diet were marginally deficient. Phosphorus fed in excess of the requirement, which in this example was close to .31%, was excreted. Referring to Figure 1, reducing P content of average U.S. dairy diets from .44-.45 to .36-.40% represents a 15% reduction in dietary P, and at least a 20% reduction in manure P.

Reducing dietary P concentration not only reduces P content of manure, but it reduces the vulnerability of P in manure from being solubilized in runoff water following field application. Ebeling et al. (2001) obtained manure from lactating cows fed dietary P concentrations of .32 or .48%. These dietary levels resulted in feces with P concentrations of .48 and 1.28%, respectively. This manure was surface applied to field plots without incorporation. Phosphorus load in water run-off from the plots was about ten times greater in plots amended with manure derived from cows fed the high-P diet than manure from cows fed the low-P diet. When these manures were applied at equivalent rates of P (36 lb/acre), the high-P manure had P runoff loads about four times that of the low-P manure. A related study was reported recently by Dou et al. (2001). They measured water solubility of P in manure obtained from cows fed different amounts of dietary P. Their study indicated that almost all of the P fed in excess of the cows requirement ended up as water soluble P in the manure. This is depicted in Figure 2. Increasing dietary P above the minimal requirement (0.3% P in this figure) did not increase P secretion in milk. Dietary P in excess of the requirement was simply excreted in the manure, and largely in water soluble form. Therefore, reducing dietary P not only reduces P content of manure, but can greatly reduce the potential for field runoff of what manure P is applied.

Table 3. Milk production response to dietary phosphorus level.

Study	Dietary P % of Diet DM		Milk Production Lb/day	
	Lo P	Hi P	Lo P	Hi P
Kincaid et al., 1981 (20 cows/trt)(10 mo trial)	.30	.54	61.6	66.0
Brintrup et al., 1993 (26 cows/trt)(two complete lactations)	.33	.39	55.9	53.9
Satter and Dhiman, 1996 (23 cows/trt)(12 wk mid-lactation)	.39	.65	52.6	53.7
Valk and Sebek, 1999				
Year 1 (6-8 cows/trt)(wk 17-37)	.28	.34	53.0	53.8
Year 2 (6-8 cows/trt)(wk 2-31)	.28	.34	75.1	72.7
Wu, Satter and Sojo, 2000 (9 cows/trt)(complete lactation)	.40	.49	80.2	79.5
Wu and Satter, 2000				
Year 1 (21 cows/trt)(complete lactation)	.31-.38 <sup>1</sup>	.44-.48 <sup>1</sup>	64.8	62.7
Year 2 (26 cows/trt)(complete lactation)	.31-.38 <sup>1</sup>	.44-.48 <sup>1</sup>	81.8	81.1
Lopez et al, 2002 (123 cows/trt)(first 23 wk of lactation)	<u>.37</u>	<u>.57</u>	<u>77.2</u>	<u>76.8</u>
Average	.34	.47	66.9	66.7

<sup>1</sup>Phosphorus content was 0.38 and 0.48% during confinement feeding for approximately two-thirds of the lactation, and 0.31 and 0.44% during grazing for the remainder of lactation for the low and high P diets, respectively.

Table 4. Performance of cows fed diets differing in phosphorus content for an entire lactation (Wu et al., 2001).

Item	Dietary P (% of DM)		
	0.31	0.39	0.47
Number of cows	10	14	13
Dry matter intake, lb/d	55.0	55.0	54.1
Milk, lb/308 d	28,684	26,200	26,677
Milk fat, %	3.64	3.50	3.64
Milk protein, %	3.16	3.13	3.10
P intake, g/d	77.5	97.5	115.6
Fecal P excretion, g/d <sup>1</sup>	43	66	88

<sup>1</sup>Estimated using 68% as the diet DM digestibility, and the means for DMI and fecal P concentrations (.538, .829, and 1.12% for the three treatments, respectively).

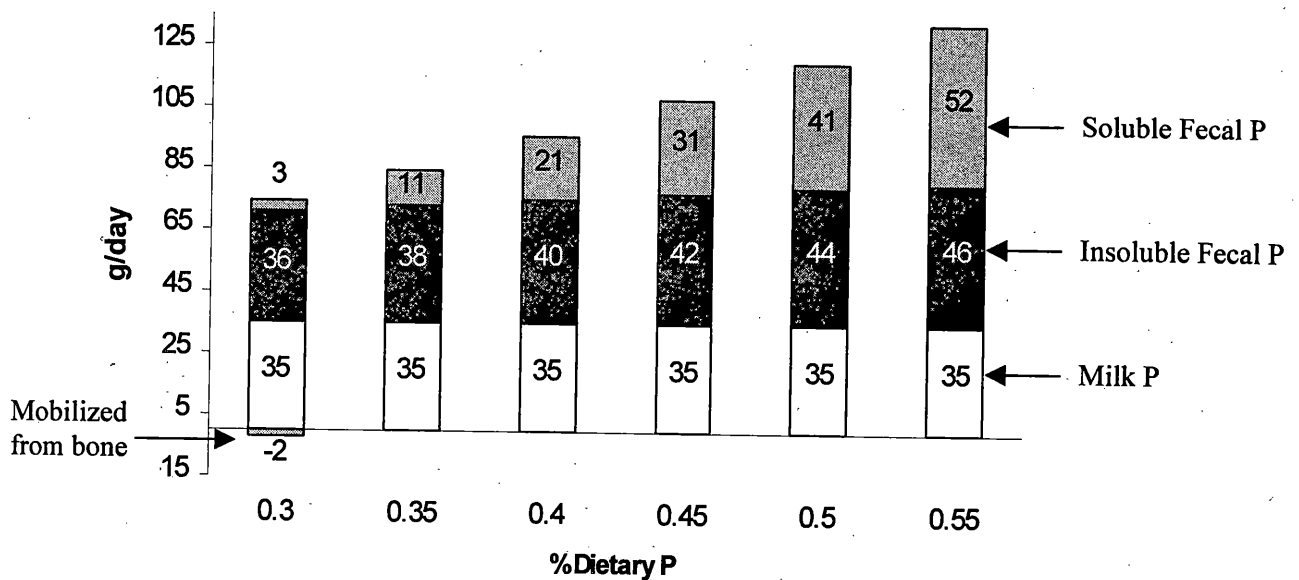


Figure 2. Destination of dietary phosphorus in a dairy cow producing 85 lb milk and consuming 53 lb diet dry matter daily. (Developed from Ebeling et al., 2001; Dou et al., 2001; and Wu et al., 2001)

Reducing dietary P can have a very significant effect on the amount of land required to effectively utilize manure P (Table 5). Most lactation diets that are not supplemented with an inorganic P source contain .35-.40% P. This of course depends upon the ration ingredients used. Since this concentration is similar to the P requirement for lactating cows, it follows that essentially all of the supplemental P fed above the requirement will be excreted in the manure. Assuming a crop uptake of 26.6 lb/acre/yr, the requirement for land increases proportionally with the increase in manure P. Reducing dietary P to an amount that the lactating cow requires often means complete elimination of mineral P supplements. It can also result in a major reduction in the amount of land required to effectively utilize manure P.

Table 5. Amount of phosphorus fed and excreted by a lactating cow producing 20,000 lb milk in 305 day, and the amount of land required to effectively utilize the manure phosphorus.

Dietary P concentration	Estimated Supplemental P	Manure P	Land area needed to recycle manure P	Change in land area
(%)	(lb/lactation)	(lb/lactation)	(acres) <sup>1</sup>	(%)
.35	0	34.8	1.3	Base
.40	7.5	42.2	1.6	23
.48	19.6	54.3	2.0	53
.55	30.1	64.9	2.4	83

<sup>1</sup>Assumptions: Cow is consuming average of 49.5 lb DM daily, and milk contains 0.09% P. There is no net change in P content of the cow. The cropping area is comprised of 37% corn for grain, 7% corn for silage, 47% alfalfa, and 9% soybeans. Crop yields are typical for the midwest US, and crops remove 26.6 lb P per acre per year.

The dairy industry utilizes large amounts of by-product feeds, many of which serve as important sources of protein in the dairy diet. There is a tendency for feedstuffs that are high in protein content to also contain high concentrations of P, but there are significant deviations from this generalization. Table 6 shows the N:P ratio of some common dairy supplements that are often brought into the ration because of their protein content. Bloodmeal and meat and bone meal represent the extremes in this table. Both feedstuffs are high in RUP content, but bloodmeal supplies a very large amount of protein per unit of P. Meat and bone meal, on the other hand, supplies relatively little protein per unit of P. For dairy producers that are having trouble managing P, choice of a protein supplement or by-product feed can be an important decision affecting P management. A growing number of dairy producers have discontinued using P supplements, but because they utilize large amounts of by-product feeds high in P concentration, overall dietary P content may still be excessive (.40-.45%). It is important that least-cost ration formulation programs do not give credit for P content of a feedstuff if the diet does not need P. A significant part of the dollar value of meat and bone meal is associated with its P content. If P is not needed, then meat and bone meal should not be given credit for the P it contributes in excess of the requirement. In fact, a negative value might be appropriately assigned in some cases.

Table 6. Protein and phosphorus content of some common feeds (NRC, 2001).

Feed	Protein Content % of DM	N content % of DM	Phosphorus content % of DM	N:P
Bloodmeal	95.5	15.3	0.30	51.0
Soybean meal (48%CP)	49.9	8.0	0.70	11.4
Soybean (Roasted)	43.0	6.9	0.64	10.8
Brewer's grains	29.2	4.7	0.67	7.0
Cottonseed	23.5	3.8	0.60	6.3
Corn distillers grains	29.7	4.8	0.83	5.8
Canola meal	37.8	6.0	1.10	5.5
Corn gluten feed	23.8	3.8	1.00	3.8
Wheat midds	18.5	3.0	1.02	2.9
Wheat bran	17.3	2.8	1.18	2.4
Meat and bone meal	54.2	8.7	4.73	1.8

Reducing dietary P in lactating cow diets is perhaps one of the most effective steps that can be taken to reduce the environmental threat of dairy manure. It is a step that reduces cost as well as provides environmental benefits. The P content of most dairy diets can be reduced by about 15%, thus lowering manure P by about 20%.

## MANAGING PHOSPHORUS FOR BEEF CATTLE

### Cow-Calf Operations

The P requirement, as well as dietary P supply, can vary greatly during the year for a beef cow. Grazing during a period of good grass growth will provide considerably more P than grazing winter pasture or corn stalks. Likewise, the requirement for P is considerably higher during lactation than after the calf is weaned. A comprehensive review of P nutrition of grazing cattle is available (Karn, 2001).

The beef NRC (1996) suggests a range of .11 to .24% dietary P (DM basis) to cover the P needs of cows consuming a wide range of diet energy densities and producing between 22 and 66 pounds of milk at peak production. The requirement is closer to the high end in early lactation, and to the low end in later lactation. Highly digestible diets should contain a little more P than low digestible diets because a little less feed dry matter will be consumed with the highly digested feed.

Performance and P status were studied in two groups of 39 range cows (Angus x Hereford) over a 5-year period in New Mexico (Judkins et al., 1985). One group had free access to salt alone (control), and the other to a mineral mix containing 50% dicalcium phosphate, 45% salt and 5%

cottonseed meal. The cows received no other mineral, protein or energy supplements during the whole trial. Lack of P supplementation had a detrimental effect on cow performance only during one year of the experiment, and that was a drought year. The combined effect of no P supplementation and drought caused the non-supplemented cows to calve later (7 April vs. 11 February) and wean lighter calves (497 vs. 557 lb) than the P supplemented cows in the year following the drought. Percent calf crop did not differ between the two groups during one year of the study. The authors concluded that rainfall (green plants have more P than dormant plants) or P supplementation before and during the breeding season may be critical in maintaining early calving date and heavier weaning weights. Other research has also shown that dietary P at approximately 65% of NRC recommendations resulted in no reduction in reproductive performance of beef females (Little, 1980).

A large experiment was done in Utah to determine the P requirements for growth and reproduction of Hereford heifers (Call et al., 1978). Ninety-six seven month-old heifers were divided into two groups, with one receiving a diet containing .14% P (as fed basis), and the other the same diet, supplemented with monosodium phosphate to give .36% P (as fed basis). The experiment lasted two years. The low P diet provided about 66% of the NRC recommended level of P and the high P diet about 174%. The average daily weight gain was .99 lb/day for both groups, and feed efficiency was similar for the two groups. The low P group had a 96% pregnancy rate with 91% live calves, and the high P group had corresponding values of 100 and 93%, respectively. These differences were not significant. After 9 months on trial (approximately 16 months of age), no differences were discernible in rib bone structure based on bone microradiographs.

While it appears that growing replacement heifers and mature beef cows will have adequate P nutrition during periods of active grass growth, it is possible that P deficiency can develop when grass quality is low, or during winter grazing of pastures or corn stalks. Since supplementing P to beef cows or beef replacement heifers is not an environmental issue, and further that with low-quality pasture or range conditions that a P deficiency could develop, it seems prudent to routinely provide free-choice phosphorus supplement to beef females grazing low-quality pastures, particularly during lactation. A free choice mixture of 50% trace mineral salt and 50% dicalcium phosphate is a good mixture to offer. Phosphorus is seldom an environmental issue with cow-calf operations, unless soil erosion is carrying P-laden soil particles from the pasture into streams and lakes. Phosphorus requirements, and hence P inputs, are low relative to dairy cows. Usually P does not accumulate in the cow-calf system.

### Feedlot Cattle

The situation regarding P supplementation to feedlot cattle is simple-DON'T DO IT. This section could be concluded with this statement, but perhaps some more information is warranted.

A number of studies have been conducted to evaluate P requirements of calves weighing less than about 400 lb (Wise et al., 1958; Miller et al., 1987; Jackson et al. 1988), but relatively little information is available for beef feedlot cattle weighing between 600 and 1300 lb. The beef NRC recommends a range of P contents-from .12% to .34% of diet DM, depending upon body weight and energy density of the diet. Erickson et al. (1999) evaluated P requirements of

yearling steers (836 lb) with typical feedlot gains of 3.3 lb/day and concluded that the requirement was less than .14% of diet DM, or 70% of the NRC (1996) recommendations. In another study with finishing feedlot calves weighing 583 lb, Erickson et al. (2002) concluded that the P requirement for finishing calves is < .16% of ration dry matter, or 14.2 g/day. Again, this is less than what the NRC would suggest for this size of feedlot animal. Since virtually all feedlot diet formulations that contain much corn grain will exceed these low requirement levels, these authors suggest that determining the P requirement for feedlot cattle is unimportant. What is important is to remove all supplemental P from feedlot diets, since they already have excess P. Supplementation of mineral P in finishing diets is an unnecessary economic and environmental cost for beef feedlot producers. Reducing dietary P to closely match the requirement would require use of very low P feed ingredients, and this would be neither practical nor cost effective.

The only information that might contradict removal of all supplemental P from feedlot diets is a recently reported study by Flatt et al. (2001). In this study involving 221 steers and heifers weighing 650 lb at the start of a 147 day feeding period, animals were either fed .34% P for the full feeding period, or were fed .34% P for the first 85 days, and then reduced to .24% P for the remainder of the feedlot period. There were no differences in growth rate, feed efficiency and carcass quality except that there were more dark cutters on the low P group (13.6% vs. 4.5%). Morbidity was also higher with the low P group (13.5% vs. 4.5%). These results are surprising and perhaps questionable, but if these observations can be repeated, then it raises another aspect to the question of supplementing P to feedlot cattle.

An emerging issue is the growing use of by-product feeds from ethanol plants in feedlot diets. These feedstuffs contain 2-3 times the amount of P that shelled corn contains. Incorporating these high P feedstuffs into the feedlot diet further increases the surplus of P in feedlot diets. These high P by-product feeds are usually economical replacements for shelled corn in feedlot rations, and producers can hardly afford not to utilize them. About all that a feedlot operation can do to minimize adverse environmental impact from the high P manure produced with such feed ingredients is to make sure that there is sufficient land upon which to spread manure, and to incorporate manure into the soil so that the soluble P will be less vulnerable to runoff.

## CONCLUSIONS

The livestock industry can do more to be good environmental stewards. One management action is to make certain that excess supplemental P is removed from our dairy and beef rations. This will not only reduce the amount of P that is excreted, it will greatly reduce the amount of soluble P in the manure. It is the soluble P that is most likely to get into lakes and streams and cause unwanted algae growth. The frosting on the cake is that producers also save money by eliminating unnecessary P supplements. If the feed industry, nutritionists, veterinarians, and producers fail to implement a practice that is a win-win situation for the environment and the producer, how is the public to interpret the livestock industry's interest in supporting the environment?

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# APPROACHES TO MEETING THE NONRUMINANT'S PHOSPHORUS REQUIREMENTS

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## INTRODUCTION

Phosphorus (P) has long been recognized as one of the most important minerals required by livestock and poultry. One of its major roles is that it complexes with calcium (Ca) to give rigidity to bones. In addition, P is an integral part of many organic compounds and it plays important roles in energy and protein metabolism. Almost every series of biochemical reactions that occurs in muscle, blood, and other soft tissues involves this important mineral.

Providing animals with adequate dietary P is not only important for the proper development and maintenance of the skeleton, but it is needed to optimize growth, efficiency of feed utilization, and lean tissue synthesis. The impact of not supplying adequate P to growing animals can be disastrous. For example, Table 1 shows the results of feeding a P deficient diet to growing-finishing pigs on growth, carcass leanness, and bone mineralization.

Table 1. Effects of phosphorus level on pig performance<sup>a</sup>

Item	Adequate diet 0.50% P	Deficient diet <sup>b</sup> 0.32% P
Daily gain, lb	1.69	1.19
Feed:gain	3.08	3.82
Carcass lean cuts, %	58.5	55.9
Bone strength, kg	145	76
Bone ash, %	57.4	52.8

<sup>a</sup>University of Kentucky experiment involving 21 pigs from 37 to 202 lb body weight.

<sup>b</sup>The low P diet consisted of corn and soybean meal without any supplemental inorganic P.

## ESTIMATES OF PHOSPHORUS REQUIREMENTS

The best source of unbiased information on the requirements for P and other nutrients for swine, poultry, and other animals is the nutrient requirement publications of the National Research Council (NRC). The NRC is part of the National Academy of Science, a private organization that was established 140 years ago to advise the nation on issues of science and technology. The NRC has established guidelines for the

feeding of domestic animals for over 70 years. Members of NRC subcommittees update estimates of nutrient requirements periodically based on new research findings. The most current guidelines were published for poultry in 1994, for swine in 1998, for beef cattle in 2000, and for dairy cattle in 2001. The current NRC estimates of the Ca and P requirements for swine and chickens are shown in Tables 2 and 3.

For many years, universities and feed companies published recommended nutrient allowances for livestock and poultry. The general approach was to use NRC standards as the base, then add extra amounts of most of the nutrients as safety factors. Little attention was paid to "oversupplementing" diets with nutrients as long as it was not overly expensive. Those nutrients that were in excess of the animals' requirements were simply stored in the body tissues or excreted in the manure.

Table 2. Calcium and phosphorus requirements of swine<sup>a</sup>

Stage	Ca (%)	P (%)	Bioavailable P (%)
Growing pigs			
3-5 kg	0.90	0.70	0.55
5-10 kg	0.80	0.65	0.40
10-20 kg	0.70	0.60	0.32
20-50 kg	0.60	0.50	0.23
50-80 kg	0.50	0.45	0.19
80-120 kg	0.45	0.40	0.15
Breeding gilts and sows			
Gestation	0.75	0.60	0.35
Lactation	0.75	0.60	0.35
Boars	0.75	0.60	0.35

<sup>a</sup>NRC (1998).

Table 3. Calcium and phosphorus requirements of chickens<sup>a</sup>

Stage	Ca (%)	Nonphytate P (%)
Meat-type broilers		
0-3 weeks	1.00	0.45
3-6 weeks	0.90	0.35
6-8 weeks	0.80	0.30
Laying hens (100 g/d of feed)	3.25	0.25
Roosters	0.90	0.45

<sup>a</sup>NRC (1994)

However, that situation has changed dramatically during the past several years, especially with respect to N and P. Environmental issues relating to water quality have forced livestock and poultry producers to pay much closer attention to their feeding programs so as to limit the amount of N and P in the manure produced by their animals.

## ENVIRONMENTAL CHALLENGES

Meeting the environmental challenges in agriculture is one of the major issues facing the animal industry. Beef cattle, dairy cattle, sheep, swine, and poultry produce nearly 160 million tons of manure annually (Sweeten, 1992; Table 4). Most of the swine and poultry waste is produced in confinement units where the nearby land base often is insufficient to accommodate the waste in an environment-friendly manner. Excess N and P in animal manure can contribute to surface and ground water pollution, and N from manure can contribute to aerial ammonia and other gasses, including those with offensive odors.

Table 4. Quantities of manure, nitrogen, and phosphorus excreted annually by livestock and poultry in the United States (dry matter basis)<sup>a</sup>

Species	Manure (million tons)	Mineral Conc. (%)		Excretion (thousand tons)	
		N	P	N	P
Ruminants					
Beef cattle	96.6	3.96	1.07	3,828	1,029
Dairy cattle	29.1	3.75	0.79	1,091	230
Sheep	1.8	3.89	0.56	70	10
Nonruminants					
Swine	15.5	4.71	2.97	730	460
Poultry	15.4	5.13	1.62	790	250
Total	158.4			6,509	1,979

<sup>a</sup>Adapted from Sweeten (1992).

Swine and poultry manures are quite high in both N and P (Table 4). The high N content is attributable to several factors. First, diets are relatively high in crude protein due to the large amounts of amino acids that are needed to support the fast growth rate in broiler chicks and the high rate of lean growth in swine. Second, the dietary protein is not completely digested as the feed passes through the digestive tract which results in approximately 15 to 20% of the dietary N passing out of the pig in the feces. Third, and probably most important, the pattern of the amino acids that are digested from the protein and absorbed into the bloodstream is far from ideal in comparison with the pattern of amino acids that is needed for growth and other functions. Thus, the N from those amino acids that are in excess of the pig's requirements is converted into urea

and excreted in the urine. The same occurs in poultry except that the excess N is excreted as uric acid.

The major reason for the high concentration of P in swine and poultry manure is that most of the P in cereal grains and oilseed meals is bound in an organic complex called *myo*-inositol 1,2,3,4,5,6-hexakisphosphate, commonly referred to as phytic acid or phytate. From 55 to 80% of the P in cereal grains and oilseed meals is in this form (Nelson et al., 1968a). In order for nonruminants to utilize the P from phytate, the phosphate groups must first be enzymatically released from the complex, a process that requires the enzyme, phytase. Unfortunately, pigs and poultry do not have sufficient amounts of phytase in their digestive tract to hydrolyze the P from phytate, so most of the P from the major ingredients in the diet (i.e., grain, soybean meal) is excreted in the feces. In contrast, ruminants utilize phytate quite well because of the abundance of phytase produced by rumen microorganisms.

### DIETS THAT REDUCE NITROGEN AND PHOSPHORUS IN MANURE

A recent publication by the Council for Agricultural Science and Technology reviewed nutritional strategies that can be implemented to reduce the N and P in manure (CAST, 2002). Feeding diets that do not have excessive levels of crude protein (amino acids) is one of the most effective means of reducing N excretion. Another effective way is to reduce the dietary protein level and add crystalline amino acids. Research at our station has shown that feeding pigs a diet with 2 percentage points less protein plus 0.15% supplemental lysine is essentially equivalent to a higher protein diet, and will reduce N excretion by 20 to 30% (Cromwell, 1996). Further reductions in dietary protein are possible if the diet is supplemented with additional lysine along with certain other amino acids such as threonine, tryptophan, and methionine. In addition, N excretion can be reduced by using high quality protein sources with good amino acid profiles, by using feed ingredients that have more highly digestible protein, and by formulating diets on an "ideal protein" basis such that those amino acids that exceed the pig's requirements are not excessive.

Similarly, P excretion can be reduced by feeding diets that do not have excessive levels of supplemental P. For example, feeding a diet with 0.2 of a percentage unit more P than needed by growing-finishing pigs (a common practice a few years ago) will result in a 70% greater P excretion compared with feeding P levels that meet NRC (1998) standards. Formulating diets on a "bioavailable P" basis rather than a "total P" basis also helps in that it allows the nutritionist to more precisely meet the P requirements without having overages of nutrients.

Several relatively new technologies now exist that have potential for reducing P excretion by nonruminant animals. One such technology is the use of phytase in diets. Another is the use of grains and oilseed meals with reduced phytate content. The rest of this paper will address these and other new technologies that have potential for reducing P excretion by nonruminants.

## PHYTASE

The enzyme, phytase (myoinositol hexakisphosphate phosphohydrolase), acts on the phytate molecule yielding ortho-phosphate and several phosphoric esters ranging from pentaphosphates to monophosphates. Generally the release order is from the 3-position initially, then from the 4, 5, 6, 1, and 2 positions in that order. Phytase is widely distributed in yeasts, fungi, and bacteria. *Aspergillus* (especially *A. ficuum*) microorganisms produce large amounts of this enzyme. In addition, some seeds (rye, wheat, triticale) have relatively high levels of phytase.

The first animal studies with phytase were conducted by Nelson and co-workers nearly 35 years ago. In their initial study, these researchers fed low-P diets containing soybean meal that had been previously treated with phytase from *Aspergillus ficcum* and reported dramatic responses in growth and bone ash (Nelson et al., 1968b). Subsequently, they added phytase to low-P diets and showed similar responses (Nelson et al., 1971). They pointed out, however, that the enzyme was much too expensive to use in practice.

The discovery of mutant strains of *Aspergillus* that produced high levels of phytase along with the advent of recombinant-derived phytase in the late 1980s and early 1990s stimulated renewed interest in phytase research. Studies by Simons et al. (1990), Jongbloed et al. (1992), Cromwell et al. (1993), Lei et al. (1993ab), and Young et al. (1993) were the first to evaluate these new forms of phytase in pigs. During the past 10 years, more than 80 refereed papers have been published in the *Journal of Animal Science* and *Poultry Science* and over 350 abstracts on phytase have been presented at the annual and sectional meetings of the American Society of Animal Science and Poultry Science Association. Most of the research studies have been conducted with Natuphos<sup>®</sup>, a commercial source of phytase marketed by BASF (Mt. Olive, NJ). This form of recombinant-produced phytase was approved for animal use by the Food and Drug Administration in 1995.

An overview of the research conducted with phytase conclusively shows that digestibility (i.e., bioavailability) of P in cereal grains and oilseed meals is markedly improved with phytase supplementation. Our studies indicate that the bioavailability of P is increased threefold, from approximately 15% in a corn-soybean meal blend to over 45% when phytase is added to the mix (Cromwell et al., 1995). This means that reduced amounts of supplemental inorganic P are needed in swine and poultry diets to maximize growth and bone mineralization. As a result, fecal P is markedly reduced when phytase is included in the diet. These effects are shown in two studies that we conducted at the University of Kentucky (Tables 5 and 6).

The effectiveness of phytase is associated with the amount that is included in the diet. Early studies by Jongbloed (1996) showed that responses in pigs maximized at approximately 1,000 phytase units/kg of diet (Figure 1). More recently, studies have shown that maximum or near-maximum responses can be achieved with lower levels of

phytase, especially, when Ca levels are also reduced in the diet. Apparently this is due to the fact that excess Ca ions tend to inhibit the activity of phytase (Lei et al., 1994).

Table 5. Effects of supplemental phytase in low phosphorus diets on performance and bone strength in finishing pigs<sup>a</sup>

Ca, %: <sup>b</sup>	0.50	0.40	0.40	0.40	0.40
P, %: <sup>b</sup>	0.40	0.30	0.30	0.30	0.30
Phytase, units/kg: <sup>c</sup>	-	-	250	500	750
Daily gain, lb	1.96	1.89	1.88	1.89	2.00
Feed:gain	3.23	3.45	3.33	3.26	3.29
Bone strength, kg	188	163	179	183	190

<sup>a</sup>Cromwell et al. (1997), University of Kentucky. The experiment involved four pens of six pigs per treatment from 125 to 243 lb body weight.

<sup>b</sup>Levels of Ca and P were 0.05% higher during the first 4 weeks of the experiment, from 125 to 175 lb body weight. The levels shown in the table were fed during the final 5 weeks, from 175 to 243 lb. All of the P in the 0.30% P diets was supplied by corn and soybean meal.

<sup>c</sup>Natuphos<sup>®</sup> (BASF, Mt Olive, NJ).

Table 6. Effects of supplemental phytase in low phosphorus diets on phosphorus excretion in finishing pigs<sup>a</sup>

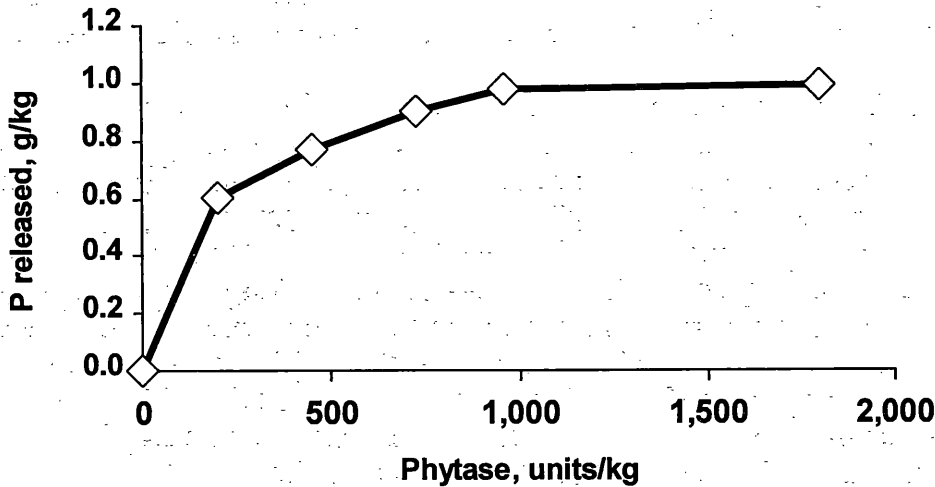
Ca, %:	0.50	0.40	0.40	0.40
P, %: <sup>b</sup>	0.40	0.30	0.30	0.30
Phytase, units/kg: <sup>c</sup>	-	-	250	500
Dietary P intake, g/day	11.17	8.43	8.53	8.27
Retained P, g/day	3.68	2.03	3.36	3.78
Fecal P excreted, g/day	7.23	6.31	4.86	4.03
Urinary P excreted, g/day	0.26	0.09	0.31	0.46
Total P excreted, g/day	7.49	6.40	5.16	4.49
Reduction in total P excretion, %	-	-	31	40

<sup>a</sup>Pierce et al. (1997), University of Kentucky. The balance experiment involved six pigs (192 lb body weight) per treatment. Feed intake averaged 5.8 lb/day.

<sup>b</sup>All of the P in the low P diets was supplied by corn and soybean meal.

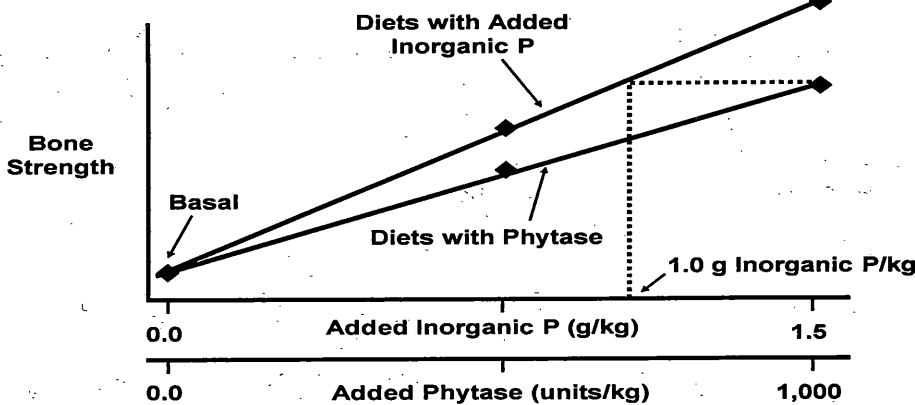
<sup>c</sup>Natuphos<sup>®</sup> (BASF, Mt. Olive, NJ).

Figure 1. Phosphorus released from phytate with various levels of phytase



How much can the P content of the diet be reduced when phytase is added? This can be calculated with regression techniques using bone traits such as illustrated in Figure 2. Early studies indicated that 1,000 units of phytase per kg of diet released approximately 1 g of P per kg of diet (1 g/kg is equivalent to 0.1 of a percentage point). Several of our initial studies supported that relationship. However; more recent tests indicate that less phytase may be required to release this amount of P from phytate if the dietary Ca level is also reduced by 0.05 to 0.10 of a percentage point. The bone strength data of Table 5 in which finishing pigs were fed diets with 0.10 point less Ca and P and various levels of phytase indicated that 650 units of phytase per kg of diet released 1 g of inorganic P per kg of diet (or 0.10 of a percentage unit of P).

Figure 2. Bioequivalency of Phytase and Organic Phosphorus



Although less clear, there is evidence that phytase increases the absorption of Ca, Mg, Zn, and other divalent cations due to the disruption of the phytic acid molecule which acts as a chelating agent (Adeola, 1996). Even less clear is the impact of phytase on amino acid availability. Some research suggests that phytase improves the ileal digestibility of amino acids (Mroz, 2002), but other research has shown no effect on amino acid utilization from phytase additions (Traylor et al., 2001; Adeola, 2002).

Recently, new forms of phytase have been developed. One such form is phytase produced by *peniophora lycci*. This is a 6-phytase, meaning that it initially removes the P from the 6 position of phytate as opposed to *Aspergillus*-derived phytase which initially removes the P from the 3 position. *Peniophora lycci* phytase is now commercially available (Ronozyme<sup>®</sup>, Roche Vitamins, Princeton, NJ). The relative efficacy of these two forms of phytase has been investigated (Augspurger et al., 2002; Lynch et al., 2002). A commercially available microbial phytase produced by solid state fermentation (Allzyme Phytase<sup>®</sup>, Alltech, Nicholasville, KY) has been shown to be effective (Wu and Ravindran, 2002). Other new forms of *Escherichia coli* phytase have recently been developed using recombinant and cloning techniques (Rodriguez et al., 1999; Leeson et al., 2000; Augspurger et al., 2002) and have been shown to be very effective.

#### LOW-PHYTATE CORN

In 1990, Raboy and co-workers identified several mutant genes in corn that suppressed the synthesis of phytic acid in the kernel without affecting the amount of total seed P (Raboy et al., 1990). In 1996, we obtained corn from Pioneer Hi-Bred International (Optimum Quality Grains/Dupont Specialty Grains, Johnston, IA) containing one of these mutant genes (*lpa1*) for animal studies. This corn contained half as much phytate P (0.10 vs 0.20%) and over three times as much inorganic P (0.18 vs 0.05%) as a near-isogenic conventional corn (Table 7). Using slope-ratio procedures, we found that the P in low-phytate corn was about three to four times more bioavailable for pigs than the P in normal corn. Specifically, the low-phytate gene increased the bioavailability of P from approximately 20% in normal corn to over 75% in low-phytate corn (Cromwell et al., 1998). Similar results were also reported by Spencer et al. (2000b) using similar procedures and by Veum et al. (2001) using an *in vitro* procedure.

Experiments with both growing and finishing pigs indicated that feeding pigs low-phytate corn-soybean meal diets containing 0.10 to 0.12% less total P than normal results in similar performance and bone mineralization as in pigs fed normal corn-soybean meal diets (Pierce et al., 1998ab). This reduction in total dietary P along with the greater bioavailability of P in low-phytate corn is associated with a 43% reduction in excreted P (Pierce, 1999), as shown in Table 8.

#### LOW-PHYTATE, LOW-OLIGOSACCHARIDE SOYBEAN MEAL

Soybeans that are low in both phytic acid and oligosaccharides have recently been produced by mutagenesis. The reason that both compounds are reduced is that the

synthesis of oligosaccharides (from soluble sugars) and phytic acid use similar metabolic pathways. The down-regulation of key enzymes in the synthesis pathways of the mutant soybeans affects the amounts of both compounds. Soybean meal produced from low-phytate, low-oligosaccharide soybeans has approximately half as much phytate P (0.22 vs 0.48%) and more than twice as much inorganic P (0.55 vs 0.22%) as soybean meal from near-isogenic conventional soybeans (Table 7).

Table 7. Composition of normal and low-phytate corn and normal and low-phytate, low oligosaccharide soybean meal<sup>a</sup>

Item	Normal corn	Low-phytate corn	Normal SBM	Low phytate SBM
	%	%	%	%
Crude protein	8.50	8.50	53.6	55.3
Lysine	0.23	0.24	3.03	3.18
Methionine	0.15	0.15	0.80	0.83
Calcium	0.01	0.01	0.35	0.36
Total phosphorus	0.25	0.28	0.70	0.77
Phytate phosphorus	0.20	0.10	0.48	0.22
Inorganic phosphorus	0.05	0.18	0.22	0.55
Carbohydrates				
Sucrose			7.22	12.32
Raffinose			0.91	0.55
Stachyose			5.20	0.53

<sup>a</sup>DuPont Specialty Grains, Des Moines, IA. The normal and low-phytate corns were near-isogenic as were the soybeans from which the soybean meals were prepared.

Slope-ratio studies conducted at the University of Kentucky have shown that the P in low-phytate soybean meal is approximately 50% bioavailable, compared with 20% in normal soybean meal (Cromwell et al., 2000a). Spencer et al. (2000a) reported similar estimates of P bioavailability in the two soybean meals.

In other studies, pigs fed diets consisting of low-phytate corn and low-phytate soybean meal with no supplemental inorganic P grew as fast and efficiently, had similar bone traits, and excreted 53% less P (Table 9) than pigs fed diets containing conventional corn and soybean meal supplemented with sufficient inorganic P to meet the pig's P requirement (Cromwell et al., 2000b).

## ENDOGENOUS AND BIOENGINEERED PHYTASE IN PLANTS

Some crops possess relatively high levels of endogenous phytase in their seeds. This was first shown by McCance and Widdowson (1944) and Mollgaard (1946) who demonstrated that wheat, wheat byproducts (bran, middlings), rye, and to a lesser extent, barley, contain significant amounts of phytase. In studies at the University of

Kentucky (Cromwell, 1993), we have found a considerably higher bioavailability of P in wheat (50%), wheat middlings (41%), wheat bran (29%), and barley (30%) than that in corn (14%) (Table 10). Wheat bran phytase has also been shown to increase the utilization of P in other feedstuffs in the diet.

Biotechnology has now been used to insert a phytase gene into alfalfa (Ullah et al., 2002) and canola (McHughen, 2000), which greatly increases their phytase content. Commercialization of such crops could be important in that it would be an alternative vehicle for supplying phytase to nonruminant diets in order to reduce P excretion.

Table 8. Phosphorus balance of finishing pigs fed corn-soy diets with normal or low-phytate corn<sup>a</sup>

	Normal corn	Low-phytate corn
Total P, %: <sup>b</sup>	0.42	0.30
Bioavailable P, %: <sup>c</sup>	0.20	0.20
P intake, g/day <sup>d</sup>	11.89	8.66
P retained, g/d	5.31	4.92
P excreted, g/day		
Feces <sup>d</sup>	5.85	3.64
Urine <sup>d</sup>	0.73	0.10
Total <sup>d</sup>	6.58	3.74
Reduction in P excretion, % <sup>e</sup>		43

<sup>a</sup>Pierce (1999), University of Kentucky. A 5-day balance experiment involving five pigs/treatment at approximately 220 lb body weight. Diets contained 0.65% lysine.

<sup>b</sup>Normal corn-soy diet contained 0.12% added P from dicalcium phosphate. Low-phytate corn-soy diet had no additional inorganic P.

<sup>c</sup>Based on the following bioavailabilities of P: normal corn, 20%; low-phytate corn, 75%; soybean meal, 25%; dicalcium phosphate, 100%.

<sup>d</sup>Normal vs low-phytate corn ( $P < 0.01$ ).

<sup>e</sup>Reduction in P excretion compared with pigs fed the normal corn-soy diet.

## TRANSGENIC PIGS POSSESSING SALIVARY PHYTASE

Scientists at the University of Guelph (Golovan et al., 2001; Forsberg et al., 2002) have recently produced several lines of transgenic pigs that have high levels of phytase in their saliva. In their studies, the true digestibility of soybean meal P by the transgenic pigs was very high (88 to 99%) and excretion of P was reduced by as much as 75% in weanling pigs (Table 11). They attributed this dramatic response to the much larger amount of enzyme continuously present in the stomach of the transgenic pig due to the copious secretion of saliva when feed is consumed. Consequently, these transgenic pigs may have delivered as much as 200,000 units of phytase to the digestive tract during the consumption of 1 kg of feed. This is considerably more than the normal

phytase supplementation of 300 to 1,000 units of phytase per kg of feed. Whether this new finding will become practical remains to be seen, but it certainly opens up a new biological approach for reducing phosphorus pollution in animal agriculture.

Table 9. Performance of growing pigs fed corn-soy diets with normal or low-phytate corn and normal or low-phytate soybean meal with varying amounts of supplemental inorganic phosphorus<sup>a</sup>.

Supplemental P, %:	Normal corn + normal soybean meal			Low-phytate corn + low-phytate soybean meal		
	0.20	0.10	0.00	0.20	0.10	0.00
Total P, %:	0.56	0.46	0.36	0.59	0.49	0.39
Bioavailable P, %: <sup>b</sup>	0.27	0.17	0.07	0.44	0.34	0.24
Daily gain, lb <sup>c</sup>	1.76	1.66	1.38	1.77	1.76	1.74
Feed/gain <sup>c</sup>	2.22	2.30	2.62	2.24	2.16	2.19
Bone traits						
Femur strength, kg <sup>c</sup>	292	219	157	313	305	292
MM strength, kg <sup>c,d</sup>	83	61	42	74	84	80
Relative strength <sup>c,e</sup>	100	74	52	98	102	98
MM ash, % <sup>c</sup>	56.5	54.2	51.7	56.5	56.9	56.2
Fecal P excretion, g/d <sup>f</sup>	7.0	6.2	5.3	5.1	4.0	3.3
Reduction in fecal P excretion, % <sup>g</sup>				27	43	53

<sup>a</sup>Cromwell et al. (2000b), University of Kentucky. The experiment involved eight pens of individually fed pigs from 49 to 108 lb body weight. Dietary lysine was 0.95% and dietary calcium was 0.65%.

<sup>b</sup>Based on the following bioavailabilities of P: normal corn, 20%; low-phytate corn, 75%; normal soybean meal, 20%; low-phytate soybean meal, 50%; dicalcium phosphate, 100%.

<sup>c</sup>Linear effect of added P in normal corn-normal soybean meal diets ( $P < 0.01$ ).

<sup>d</sup>Average of third and fourth metacarpal and metatarsal bones.

<sup>e</sup>Relative to the normal corn diet with the highest level of P. Mean of femur and MM.

<sup>f</sup>Linear effect of added P in both diets ( $P < 0.01$ ).

<sup>g</sup>Reduction in P excretion compared with pigs fed the normal corn-normal soy diet with 0.56% total P.

## SUMMARY

Several technologies are available to nutritionists to formulate low-P, environmentally friendly diets for swine and poultry. Balancing diets on a "bioavailable P" basis (or a "nonphytate P" basis) is an effective tool to more precisely meet P requirements of nonruminants. An appropriate reduction in the dietary P level and the addition of a phytase supplement is presently one of the most effective strategies that can be used. As low-phytate corn and other grains become commercially available, their use will also provide further avenues for reducing P in swine and poultry manure. Eventual commercialization of low-phytate soybean meal will be an additional bonus. Transgenic crops with cloned phytase genes are on the horizon, and pigs having high levels of

phytase in their saliva may someday be practical and accepted. These and other new technologies provide exciting times for the future of animal agriculture.

Table 10. Bioavailability of phosphorus in cereal grains, grain byproducts, high protein meals, and inorganic phosphates for pigs<sup>a</sup>

Feedstuff	Bioavailability of P, % <sup>b</sup>	Feedstuff	Bioavailability of P, % <sup>b</sup>
<b>Cereal grains</b>		<b>High protein meals - plant origin</b>	
Corn	14	Cottonseed meal	1
Grain sorghum	20	Sunflower meal	3
Oats	22	Peanut meal	12
Barley	30	Canola meal	16
Triticale	46	Soybean meal, dehulled	23
Wheat	50	Soybean meal, 44% CP	31
Corn, high moisture	53	Soybean meal, low-phytate	50
Corn, low-phytate	75		
<b>Grain byproducts</b>		<b>High protein meals - animal origin</b>	
Oat groats	14	Meat and bone meal	90
Hominy feed	14	Dried skim milk	91
Corn gluten meal	15	Dried blood meal	92
Rice bran	25	Fish meal	93
Wheat bran	29	Dried whey	96
Brewers dried grains	33	<b>Inorganic phosphates</b>	
Cooked cereal fines	40	Steamed bone meal	82
Wheat middlings	41	Defluorinated phosphate	97
Corn gluten feed	59	Monocalcium phosphate	100
Distillers grains plus solubles	76	Dicalcium phosphate	100
<b>Miscellaneous</b>		<b>Phytase addition</b>	
Soybean hulls	78	Corn-soy diet without phytase	15
Alfalfa meal	100	Corn-soy diet with phytase	45

<sup>a</sup>Based on University of Kentucky research.

<sup>b</sup>Relative to the bioavailability of phosphorus in monosodium or monocalcium phosphate, which is considered to be 100% bioavailable.

Table 11. Ability of transgenic pigs possessing high salivary phytase to utilize phytate phosphorus<sup>a</sup>

Item	Control pigs	Transgenic pigs
Median salivary phytase, units/ml	0	2,420 <sup>b</sup>
True digestibility of soybean meal P, %		
Weanling pigs	49	88
Growing-finishing pigs	52	99
Fecal P, % of dry matter		
Weanling pigs	3.4	0.8
Growing-finishing pigs	3.0	1.3

<sup>a</sup>Adapted from Golovan et al. (2001), University of Guelph.

<sup>b</sup>Saliva from transgenic pigs ranged from 341 to 10,077 phytase units/ml.

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# ESTABLISHING NATIONAL STANDARDS FOR ESTIMATING NUTRIENT EXCRETIONS

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## INTRODUCTION

Over-application of any source of fertilizer nutrients will likely promote losses that threaten environmental quality, especially with respect to water quality. For this reason, livestock producers are often held accountable for their manure nutrient production and utilization. Accountability requires that a producer formulate a nutrient management plan (Van Horn et al., 1991, 1996, 1998a, 1998b). The plan includes number of animals to be produced, estimated nutrient excretion in manure, manure nutrients recovered and applied for fertilizer (based on manure analyses), and a plan to export nutrients off-farm if there is excess manure production relative to on-farm crop production needs. A more detailed overview of the whole-farm planning process is described elsewhere (Powers and Van Horn, 2001). Actual farm data and analyses are necessary for accurate nutrient budgeting for that farm, however, published book values provide helpful estimates of manure nutrient excretions, nutrient losses, and crop removals to use in planning new facilities or until accurate farm data are generated. Most frequently, table values are used to estimate nutrient excretions. However, these values are often outdated, not representing modern animals and production practices, and do not allow for site-specific management practices to be incorporated. A mass balance approach that considers animal diet and performance, proven to be an accurate means of predicting manure excretion composition and quantity (Tomlinson et al., 1996; Beede and Davidson, 1999), offers the advantage of tailoring a plan to reflect individual farm characteristics.

The mass balance approach does not reflect nutrient transformations that occur post-excretion but can serve as a check to determine if recovered manure reflects feasible losses. The purpose of this paper is to illustrate how a nutrition-performance approach can be included in a nutrient management plan and to discuss a national initiative to use such an approach as the foundation for revision of the American Society of Agricultural Engineers Standard D384.1 *Manure Characteristics and Composition* (ASAE, 1994).

## THE NUTRITION-BASED MODEL APPROACH TO EXCRETION PREDICTION

Manure is what is excreted in the form of urine and feces after the animal has digested and utilized all that it is going to from the ration provided plus the endogenous losses from various metabolic processes. By definition, apparent digestibility is considered to be the difference between amount consumed and amount collected in feces. Previous nutrition research has given

us good estimates of apparent digestibilities of ingredients that can be combined to estimate total ration digestibilities.

Knowing digestibility and, hence, indigestibility of the ration DM and organic matter (OM) plus quantity consumed permits us to estimate the amounts of DM and OM excreted, components that determine manure volume. Urine is the avenue of excretion for several metabolic end products, most importantly urea (uric acid for birds) with respect to nutrient management. Urine contributes significantly to wet manure weight or volume, 30 to 50%, but contributes much less to dry volume, 10 to 15% (Morse et al., 1992; Tomlinson et al., 1996). Urine, however, is the major excretion pathway for rapidly available fertilizer-N (urea or uric acid), elemental potassium (K), and sodium (Na). Excreted elemental phosphorus (P), calcium (Ca), and slower-released N from undigested protein primarily are in feces (Morse et al., 1992; Tomlinson et al., 1996).

If animals are consuming dietary nutrients at maintenance levels, e.g., N, P, and K, they will excrete, on-average over time, the same amount of N, P, and K they consumed except for small amounts of nutrients in shed hair and sloughed tissues that usually are collected with manure. Maintenance needs and equivalent excretions for those nutrients will be accounted for by turnover, without net gain or loss, of nutrients in current body contents. When animals are accumulating N, P, and K in body weight gain, offspring, milk, eggs, or wool (products), the amount of those nutrients excreted in manure (feces plus urine) differ from what is fed by the amounts in products produced. Thus, nutrient content of intake coupled with good estimates of the content of the same nutrients in food products leaving the farm permit accurate estimation of total nutrient excretions in feces plus urine by difference (Tomlinson et al., 1996; Watts et al., 1994; Patterson and Lorenz, 1996).

Table 1 presents a nutrition-based approach to estimating manure N, P, and K excretions based on ration content less amounts estimated to be in milk, eggs, or animal gain (from Powers and Van Horn, 2001). This table was derived from a spreadsheet-based model developed by Powers and Van Horn (2001) using a 'feed input minus excretion in product' approach shown by others to accurately predict excretions (Tomlinson et al., 1996, McGahan and Casey, 1998; Beede and Davidson, 1999). Product compositions were obtained using published book values; composition of milk, weight gain, eggs, and wool are minimally affected by diet. Rather, quantity of product is a reflection of nutrient intake. It should be noted that although product compositions are species-specific, weight gain for broilers, turkeys, and pigs is averaged over a life cycle grow-out for each species so at any point in that cycle the actual composition might vary from values recorded in table 1. Also, it should be noted that P and K excretion estimates here and throughout this paper are actual P and K and not  $P_2O_5$  or  $K_2O$  as used in fertilizer nomenclature. The same principles can be used to estimate content of many other dietary elements but discussion here will focus on N, P, and K, the major fertilizer nutrients other than Ca, with N and P the major nutrients of environmental concern. Use of this type of nutrient excretion prediction has proven to reflect actual P excretion more closely than other available models (Beede and Davidson, 1999).

The rations shown in table 1 for the different food animal species, i.e., dry matter intake (DMI) and content of crude protein, phosphorus, and potassium, are representative of rations fed to these animals nationally to produce expected yields of milk, eggs, and body weight gain (kg/d) for dairy cows, hens, and beef steers and the gain/life cycle grow-out for broilers, turkeys, and pigs. Note that production units for hens in table 1 are per 1000 hens. Calculations within the table predict amounts consumed of N, P, and K, the amounts in exported food animal products such as milk, eggs and live animal gains, and, by difference, the amounts excreted in manure.

Variation in nutrient intake by animals is the most important single contributor to variation in nutrient excretions (Tomlinson et al., 1996; Morse et al., 1992). Utilization of an input-output model as used in table 1 and by others (McGahan and Casey, 1998) adjusts for the variation in intake and the amounts of nutrients that are converted to the products produced. As an example, excretions by a dry cow and an early lactation cow producing 45 kg of milk per day varied from 4.5 kg to 9.8 kg of DM/d (DM equals total solids, TS), from .165 to .467 kg N/d, and from .046 to .094 kg P/d (Van Horn et al., 1994). These differences were expected and predictable based on ration parameters and performance. Although widely used excretion estimates such as *ASAE Standards* (1994) fall in the cited ranges, the estimates tend to be for average performance, are not farm specific, and do not provide a method for producers to evaluate consequences of overfeeding given nutrients. Similarly, with poultry, Patterson and Lorenz (1996) utilized an input-output method to predict excretions because they found in their extensive 2-yr field study on eight commercial Leghorn layer flocks that today's hens produce less manure than older literature values imply.

Using nutrition based input-output methods to estimate solids and nutrient excretions when nutritional data are available eliminates most of the variation in estimated manure production found in the literature and the method permits calculating what the effects of dietary changes would be on excretions. Nutrition managers on large animal-food production units, who have computerized records of feed nutrient deliveries to animals, can provide key nutrient intake information to tailor nutrient excretion estimates to actual input-output data for a specific farm. Records of food product sales off-farm along with measured or estimated nutrient content of the products provide the other component needed to accurately estimate total manure nutrient excretions.

One reason grazing budgets are so important is that manures exported off the farm often are used on pastures for beef production, especially poultry manures in intensive poultry production areas in the South and Southeast. Total nutrient budgets for pasture conditions seldom show excessive nutrient applications from manure distributed by grazing animals. If commercial fertilizer nutrients are applied in addition to manure from grazing animals or if pastures accommodate a relatively large number of cattle being fed primarily from feed sources obtained off-site, nutrient applications, especially P, may become greater than removals.

Estimating nutrient excretions of cattle on pasture is more difficult than cattle confined in barns or lots because DM and nutrient intakes from pasture are very difficult to quantify. Estimates of intake, as a percent of BW, can be made if pasture digestibility is measured and monitored.

Powers and Van Horn (2001) provide data demonstrating use of this approach to estimate excretions from the grazing animal.

Reviewing pasture budgets for beef and dairy cattle (Kunkle, 1994 and Van Horn et al., 1996) show that supplemental N fertilization from commercial fertilizer, or from N fixation via legumes, is necessary to prevent N depletion and to maintain forage production even with dairy cows fed more than half their DMI from imported concentrates. The case for P, however, shows that accumulation is likely, even when no commercial fertilizer P is applied to the pastures. Therefore, use of P fertilizers on pastures should be avoided after soil storage levels reach desired fertility levels and dietary P concentrations should be held to the minimum needed for optimum performance.

Literature confirms a large variability in manure nutrient content. Manure N content from mature milking cows averaged 5.05% (Safley et al., 1986). Tomlinson et al. (1996) showed that diets containing 12%, 15%, or 18% crude protein (CP) on a DM basis yielded urine and fecal N excretions that combined to produce manures containing 3.2%, 4.2%, and 5.3% N of total manure DM; a second study with 14 and 18% CP diets produced manures with 4.3 and 5.3% N (DM basis). Other estimates that are somewhat lower (3.78%, 3.96%, and 4.0% from *ASAE Standards*, 1994, MWPS-18, 1985, and USDA-SCS, 1992) suggest that samples either were from cows fed much lower dietary protein than used in table 1 calculations, were not fresh manures, or some urine separated and was not with feces in proportions excreted by cows.

Phosphorus content of dairy manure samples averaged 0.82% (Safley et al., 1986). Other tables cite similar values (0.80, 0.70, and 0.74% from *ASAE Standards*, 1994; MWPS-18, 1985; and USDA-SCS, 1992). Morse et al. (1994) observed a mean P content of 0.67% but manure P concentrations of dry matter were 0.42, 0.53, and 0.81% when diet dry matter contained 0.31, 0.42, or 0.54% P (calculated manure compositions from P excretion data in Morse et al., 1992b and average manure dry matter excretion reported in Morse et al., 1994). As is the case with N, P excretion is primarily dependent on P intake and P in products exported (e.g., table 1). The expected P content of manure for the dairy ration and production scenario in table 1 was 0.93% of excreted manure dry matter and 1.16% of collected manure (20% dry matter reduction). Recall, values in table 1 reflect average diets fed nationally.

#### ESTIMATION OF POST-EXCRETION COMPOSITIONAL CHANGES

Nutrition based models predict the amounts of nutrients in fresh manure excretions more accurately than collections from animal pens because of the dynamic state of manure after excretion. For example, usually 40 to 50% of the excreted N will be in urea or uric acid in the urine component for ruminants (Tomlinson et al., 1996) and up to 75% for swine (*ASAE Standards*, 1994; Carter et al., 1996). Urease enzyme, that is of bacterial origin and is nearly ubiquitous in the environment, converts urea and uric acid N to ammonia that can be lost to the atmosphere. Also, anaerobic digestion that begins in the large intestine of animals before feces are voided, continues after excretion if environmental conditions permit. Or a shift to oxidative fermentation may take place, e.g. composting and degradation on soil surfaces. Either way,

volume reduction takes place as carbon compounds are emitted to the atmosphere, primarily carbon dioxide from aerobic degradation or methane and carbon dioxide from anaerobic degradation. Additionally, variation in composition of manure collected occurs because physical separations may take place in animal pens and within the manure management system. For example, urine or urine plus added water may drain away from fecal residues thus making solids collected from animal pens different from original excretion. Some systems deliberately separate solids. Measures of both excreted and collected amounts are both important because differences give estimates of losses that occurred after excretion, which are site-specific losses. Also, due to sampling error that occurs in a manure storage structure, excretion estimates become very useful in determining content of nonvolatile components such as P and K.

Using the input-output method to predict manure nutrient composition on a dry basis (table 1) suggests that there is less variation in freshly excreted manure composition than usually reported in collected manures. It is much more difficult to predict either the amounts or the composition of manure that is recovered for use because of many differences from farm to farm in manure management procedures. Stored manure amounts and composition vary with manure handling system and housing. Feed and bedding spilled into the manure collection areas contribute to variability. Faulty watering facilities which drip or overflow dilute manure solids and nutrient content and as does moving manure from housing facilities to storage facilities by flushing alleys with water. Nutrient losses occur during manure storage and treatment, especially volatilization of ammonia and the amount volatilized varies with type of storage (covered vs. uncovered, stirred) and pH. Manure mineral content will vary in response to dietary inputs as well. Macro- and micro-mineral contents of manures reflect the dietary levels of these elements (Morse et al., 1992).

#### ADVANTAGES OF THE INPUT-OUTPUT MODEL

The major advantage of showing that manure nutrient production is a function of ration and performance (table 1) is that it is easy to visualize the importance of ration management to minimize excretions. For example, supplementation of limiting amino acids permits reduction of total dietary protein and, hence, reduces excretion of N (e.g., Carter et al., 1996). For every percentage unit that dietary protein can be reduced, table 1 calculations predict that excretion of N by different species would be reduced by 8 to 10% (average of 8.5%) which would reduce manure N to manage. Reducing dietary crude protein percentage (CP) for dairy cows from 18 to 15 to 12% reduced urinary N excretion from 228 to 138 to 99 g/d while fecal N was reduced from 199 to 179 to 158 g/d (Tomlinson et al., 1996). By reducing urea (urinary) excretion, the percentage of excreted N lost to ammonia volatilization also will be reduced.

Surveys indicate (e.g., Shaver and Howard, 1995; Watts et al., 1994) that dairy and beef producers usually feed more dietary P than animals require (e.g., NRC, 1989, for dairy cattle) and, thus, excretions can be reduced by dietary reduction. For example, if ration P as percent of dry matter were reduced 0.1% in all rations in table 1, a 12-25% relative reduction in ration P, the amounts of P in manures from confined livestock operations nationally could be reduced by 193,000 Metric tons. Preliminary analyses of current USDA research with dairy cattle (Satter et al., 1997) suggests that it may be possible to reduce P content to 0.35% of dietary dry matter,

below the currently accepted dietary requirement (National Research Council, 1989), without detriment to the animal. Changing the P content of ration dry matter for the average dairy cow in table 1 to 0.35% of dietary dry matter lowers estimated P excretion from 81 g/d to 48 g/d, changes estimated P% in manure excreted from 0.93% to 0.56% of DM and P% in manure DM collected from 1.16% to 0.69%.

## A NATIONAL INITIATIVE TO UPDATE THE ASAE STANDARD

For the past year, agricultural engineers and animal scientists have been working together to update the current ASAE Standard D384.1 such that it will reflect modern animal production practices. Recent environmental and regulatory emphasis on nutrient issues requires that this standard be useful in developing site-specific Comprehensive Nutrient Management Plans. A key component to such a plan is the integration of animal feeding practices into manure mass and nutrient excretion and reasonable estimates of nutrients removed from manure storages.

A joint Federation of Animal Science Societies (FASS) and ASAE committee was established in fall 2001. Overarching goals of the committee were identified:

- **As Excreted - Feed Intake Summary:** Characteristics of excreted manure will be defined based upon a mass balance approach using estimates of feed intake and animal retention and calculation of excretion by difference or other appropriate relationships.
- **As Excreted – Average Summary:** A review and modification of the existing ASAE D384.1 tables will define characteristics of excreted manure for typical feed programs.
- **As Removed – Average Summary:** An update or modification of MWPS-18 (Section 1; 2000) on Manure Characteristics will summarize typical manure characteristics as removed from common animal housing and manure storage systems.

Working groups were formed and involvement from additional scientists solicited. To date, over 30 scientists are involved in this activity. Working groups set forth goals and objectives based on the assessed availability of data and anticipated needs for the respective industries. Tailored approaches to addressing the objectives of the activity have been developed by each working group.

## CONCLUSIONS

Nutrient management planning is an essential component for management of livestock operations. To develop plans that adequately reflect practices of an operation, an accurate method to estimate nutrient flows, including nutrient excretions, is needed. Predicting nutrient excretions can best be accomplished by using a mass balance approach, which considers nutrient inflow, via dietary intake and nutrient outflow via absorption and utilization (in the form of product: milk, growth, eggs). This approach also serves as a check for manure sampling to determine if calculated losses and recoveries are feasible. A current national initiative is moving current table

values, non-specific towards operational differences, in the direction of using a mass balance approach to estimate nutrient excretions. Producers interested in employing this approach in their whole-farm nutrient plan should include a nutritionist on their nutrient management team in order to provide a nutrition-based estimate without compromising animal performance.

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Table 1. Estimates of N, P, and K excretions based on ration and products produced (from Powers and Van Horn, 2001).

Herd or Flock information	Units	Numbers below expand from daily averages to years			Numbers below based on life cycle grow-out		
		Dairy cows	Beef steer	Hens	Broilers	Turkeys	Pigs
Animals/day or animals/grow-out	No.	1	1	1000	1	1	1
Average DMI kg/day =	kg	21.8	9.53	94.8	3.91	24.3	298.0
Average diet crude protein (CP) % (DM basis) =	%	17.0	12.0	16.4	20.0	20.0	16.5
Average diet N % = CP % x .16 =	%	2.72	1.92	2.62	3.20	3.2	2.64
Average diet total P % (DM basis) =	%	0.50	0.40	0.65	0.80	0.80	0.57
Average diet K % (DM basis) =	%	1.20	0.80	0.60	0.60	0.60	0.66
Milk yield or egg yield kg/d =	kg	27.2		47.6			
Milk or egg protein percentage	%	3.2		10.4			
Milk or egg N%	%	0.496		1.664			
Milk or egg P%	%	0.10		0.21			
Milk or egg K%	%	0.15		0.12			
Average net body weight gain kg/day or grow-out	kg	0.09	1.41	0.839	2.18	10.80	115.2
Average N % of weight gain	%	1.20	1.60	2.20	2.60	2.10	2.32
Average P % of weight gain	%	0.70	0.70	0.60	0.60	0.60	0.72
Average K % of weight gain	%	0.20	0.20	0.20	0.20	0.20	0.20
Average diet DM digestibility %	%	65	80	83	84	82	82
Ratio: Feed DM:(milk, doz eggs, or gain)	Ratio	0.80	6.76	3.16	1.79	2.25	2.59
<u>Daily or grow-out balances<sup>1</sup>:</u>							
Nitrogen (N):							
Input: g DMI x N/DMI =	g	593	183	2488	125	778	7867
Export: g milk or eggs x N% =	g	135		793			
g gain x N/gain =	g	1	23	18	57	227	2673
Difference (manure estimate) = input - export	g	457	160	1677	68	551	5195
Yearly or grow-out manure N =	g	166764	58552	611946	68	551	5195
Phosphorus (P):							
Input: g DMI x P/DMI =	g	109	38	616	31	194	1699
Export: g milk or eggs x P% =	g	27		100			
g gain x P/gain =	g	1	10	5	13	65	829
Difference (manure estimate) = input - export	g	81	28	511	18	130	869
Yearly or grow-out manure P =	g	29621	10311	186569	18	130	869
Potassium (K):							
Input: g DMI x diet K%/100	g	262	76	569	23	146	1967
Export: g milk or eggs x K%/100	g	41		57			
g gain x K%/100	g	0	3	2	4	22	230
Difference (manure estimate) = input - export	g	221	73	510	19	124	1736
Yearly or grow-out manure K =	g	80518	26798	186138	19	124	1736

<sup>1</sup>Expanded from daily averages above to annual or life cycle grow-out balances

# DRY MATTER INTAKE OF LACTATING DAIRY COWS

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## INTRODUCTION

All species require specific amounts of nutrients for maintenance, growth, production and reproduction. Accurate determination of dry matter intake (DMI) is essential to ensure adequate amounts of nutrients are supplied to the animal. The exact mechanism that regulates feed intake is not completely understood. However, the interaction of physical dietary characteristics and chemical digestion end-products have a large effect on DMI (Allen, 2000).

Two theories of feed intake regulation exist: (1) intake is restricted due to physical fill or distension of the reticulo-rumen or (2) intake is regulated by a satiety signal based on absorption of fermentation acids. Both theories are dependent on the diet consumed.

## PHYSICAL REGULATION

Physical regulation of DMI occurs when diets high in forage are fed or when mature, low digestibility forages or feeds are fed. Both of these situations lead to increased chewing time and distension of the gastrointestinal tract (Allen, 2000). Distension of the reticulo-rumen is determined by weight and volume of digesta in the rumen. Particles that require additional rumination time due to size or digestibility may have a negative impact on DMI. To maintain DMI, indigestible particles must be removed from the rumen. Therefore, the physical regulation of DMI is determined by the interaction of the feed or fiber digestibility and particle size.

Cell wall concentration is an indicator of rumen fill. According to Jung and Allen (1995), cell wall concentration is negatively related to the intake of ruminants consuming high forage diets. Large differences in fiber digestibility exist among plant species due to the proportions of cell wall components and their structure. Grasses generally contain greater concentrations of hemicellulose and less lignin than legumes. However, due to cell wall structure, grasses are more slowly digested but to a greater extent than legumes. Legumes typically have greater lignin concentrations. However, legumes contain pectin, a rapidly digested soluble carbohydrate, as one of the cell wall constituents. The structure of cells that make up the stem of legumes are arranged in an order that increase accessibility of rumen microbes. Therefore, rate of digestion is more rapid than grasses yet extent of digestion is less.

Khrasani et al. (2001) compared alfalfa silage and bromegrass silage fed at 50:50 or 65:35 forage to concentrate (F:C) ratios. Rate of DM degradation for alfalfa silage was significantly faster than the bromegrass silage (4.70 vs. 2.50 %/h, respectively). However, DM digestibility was significantly lower for the alfalfa silage than the bromegrass silage (38.7 vs. 56.0 % of total DM, respectively). Dry matter intake was not significantly affected by silage treatment but tended to be greater for 50% compared to 35% concentrate diets. Rumen fill was significantly greater for the bromegrass silage diets. Milk production was not reported in this study. Oba and Allen (1999) evaluated the importance of neutral detergent fiber (NDF) digestibility from forage on DMI and milk production. When evaluating NDF digestibility across all forage species (legumes

and grasses), low NDF digestibility (legumes) resulted in higher DMI and milk production than the high NDF digestibility diets (grasses). In the studies comparing grass and legume NDF digestibility, grasses were higher in NDF digestibility than legumes but due to particle fragility and shorter rumen retention time, legume diets resulted in greater milk production (Allen, 1996; Hoffman et al., 1998).

During diet formulation, a minimum level of dietary neutral detergent fiber (NDF) is often set as a constraint to ensure adequate amounts of fiber for good rumen function while alleviating physical fill limitations. The 2001 Nutrient Requirements of Dairy Cattle recommends minimum concentrations of dietary and forage NDF as well as maximum concentrations of non-fibrous carbohydrates (NFC) as shown below. The recommendations are based on adequate diet particle size but do not contain adjustments for diets with increased NDF digestibility. Quantifying the production effects of increased NDF digestibility is difficult because of differences in experimental design, treatments, analysis, and growing conditions.

Table 1. Recommended minimum concentrations (% of DM) of total and forage NDF and recommended maximum concentrations (% of DM) of NFC for diets of lactating cows when the diet is fed as a total mixed ration, the forage has adequate particle size and ground corn is the predominant starch source. Nutrient Requirements of Dairy Cattle. 2001. Table 4-3, pg 37.

Minimum Forage NDF, % of DM	Minimum Dietary NDF, % of DM	Maximum Dietary NFC, % of DM	Minimum Dietary ADF, % of DM
19	25	44	17
18	27	42	18
17	29	40	19
16	31	38	20
15	33	36	21

#### DIET DRY MATTER CONTENT

Since the acceptance of total mixed rations (TMR) and the increasing use of ensiled forages, the effect of dietary moisture content on dry matter (DM) intake has been studied. Studies reviewed by Chase (1979) indicate a negative relationship between DMI and diets high in moisture content. A decrease in total DMI of 0.02% of body weight for each 1% increase in moisture content of the diet above 50% was indicated when fermented feeds were included in the ration. A 1983 study by Lahr et al. compared feeding an identical diet differing only by the addition of water to adjust TMR DM content. Treatments were 78, 64, 53, or 40% DM. Authors concluded an optimum range for dietary DM to maximize DMI was between 65 and 75%, which is well above the findings of Chase. On the other hand, Kellems et al. (1991) fed identical diets that only differed in DM (62.7, 52.8 and 45.8%) from inclusion of different DM of the alfalfa silage and found a trend toward reduced DMI as diet DM decreased. Milk production was also lower for cows fed the low DM diet compared with cows fed the high DM diet and intermediate DM diet, 35.7 vs. 37.2 and 37.0 kg/d, respectively. From these two studies we can conclude that either extreme in dietary DM has a significant effect on DMI. An optimum range may be greater than 53 and less than 75% DM.

Although the above studies are very conclusive in their findings, it is more typical to see rations with a diet DM between 50 and 60%. This may be due to the increasing use of corn silage in

today's rations compared with previous studies. Another factor is the increased acceptance and use of all ensiled forages and wet co-products in today's rations. Unfortunately, there is not a comprehensive database to make recommendations for diets that are too dry.

### CHEMOSTATIC FACTORS

Lactating dairy cows are typically fed highly digestible diets composed of non-forage fiber sources, high quality forages and high energy concentrates. Another theory of feed intake regulation is based on satiety signals caused by the absorption of fermentation acids through the rumen wall. Because DMI decreases as dietary forage increases, it has been hypothesized that acetate signals satiety. A study was conducted by Shepard and Combs (1998) to isolate the effects of acetate, propionate and NDF on feed intake of lactating dairy cows. Dietary treatments included a low NDF diet at 40:60 F:C (LF), a high NDF diet at 60:40 F:C (HF), high NDF diet + acetate infusion (HFA), and high NDF diet + propionate infusion (HFP). Quantity of infusate was based on the difference in energy supplied between the HF and LF diet. Researchers reported an increase in DMI for the LF vs. HF diet (26.3 vs. 25.7 kg/d, respectively). However, the HFA and HFP diets both resulted in a significant reduction in DMI compared with the HF diet. The HFP diet further depressed intake compared with the HFA diet (23.5 vs. 24.9 kg/d, respectively). The authors concluded that factors beyond NDF intake and acetate production regulate feed intake. And that absorbable energy, specifically propionate, has a significant impact on DMI.

### ENVIRONMENTAL EFFECTS

The effect of environment temperature and humidity on dry matter intake (DMI) and milk production of lactating dairy cows has only been studied to a limited extent (NRC, 1987; NRC, 2001). Most previous research has focused on the effects of heat stress or environmental conditions above the thermal neutral zone of 5 to 20° C. McGuire et al. (1989) measured DMI and milk yield of Holstein cows in a thermal comfort zone of 19 to 25° C and a thermal stress zone of 19 to 40° C. Cows in the thermal comfort zone ate 25% more DM (15.1 vs 11.1 kg/d) and produced about 3 kg/d more milk (19 vs 16.2 kg/d) than cows in the thermal stress zone. Restricting DMI of cows in the thermal comfort zone to 75% of ad libitum DMI mimicked the effects of thermal stress.

West et al. (1999) studied the interaction of environmental conditions and four concentrations of NDF in the diet on DMI of lactating Holstein cows. They found as the environmental temperature and humidity increased and the NDF concentration of the diet increased, DMI of cows decreased linearly. The decrease in DMI was greatest (22%) for cows fed the low NDF (30% of the DM) and least (14%) for cow fed the highest NDF diet (42% of DM) as the environment changed from less than 72 temperature humidity index (THI) to over 78 THI. Holter et al. (1997) reported a similar decrease (22%) in DMI of mid to late pregnant multiparous Holstein cows subjected to heat stress conditions. Eastridge et al. (1998) suggested the following change in DMI occurs when temperatures are above 20° C:  $DMI (kg/d) \times (1 - ((\text{ }^\circ\text{C} - 20) \times .005922))$ .

Very limited information is available on the change in DMI when cows are in environmental conditions below the thermal neutral zone. In the 2001 Nutrient Requirements of Dairy Cattle (NRC, 2001) temperatures below the thermal neutral zone are suggested to alter nutrient

metabolism and increase maintenance requirements. Eastridge et al. (1998) suggested the following change occurs in DMI of lactating dairy cows when environmental temperatures decrease below 5° C:  $DMI (kg/d)/(1 - ((5 - ° C) \times .004644))$ .

### DRY MATTER INTAKE PREDICTION EQUATIONS

Several equations have been developed to predict the DMI of lactating dairy cows over the past 25 years. In 1994, May summarized equations most commonly used up to that date. He observed that there are 2 types of DMI equations developed 1) equations that predict maximum DMI, 2) equations that predict expected DMI. Table 2 contains prediction equations published or evaluated since 1994.

Table 2. Dry matter intake prediction equations published or evaluated since 1994.

Equation	Intake equation*	Reference
[1]	$DMI = 0.008037BW + 0.3134FCM + 0.2286DIM - 0.002176DIM^2 + 0.00000705DIM^3$	Kertz et al. 1991; overall
[2]	$DMI = 0.007075BW + 0.3005FCM + 0.272DIM - 0.001943DIM^2$	Kertz et al. 1991; multiparous
[3]	$DMI = 0.011588BW + 0.2608FCM + 0.1607DIM - 0.000955DIM^2$	Kertz et al. 1991; primiparous
[4]	$DMI = 0.95931 + 1.05134WOL - 0.04163WOL^2 + 0.00051WOL^3 + 0.01198BW + 0.35409FCM - 1.96552MF + 0.94075MP$	May 1994; multiparous
[5]	$DMI = -2.11995 + 0.88242WOL - 0.03108WOL^2 + 0.00033WOL^3 + 0.01647BW + 0.35081FCM - 1.50828MF + 0.75248MP$	May 1994; primiparous
[6]	$DMI = (0.372FCM + 0.0968BW^{0.75}) * (1 - e^{-\frac{0.192 * (WOL + 3.67)}{0.192 * (WOL + 3.67)}})$	NRC 2001
[7]	$DMI = -0.293 + 0.0968BW^{0.75} + 0.372FCM$	Rayburn and Fox, 1993
[8]	$DMI = (8.4 + 0.006BWC + (12.2 * MPY)) * Lag^{**}$	Roseler et al. 1997; multiparous
[9]	$DMI = (4.6 + 0.011BWC + (12.4 * MPY)) * Lag^{***}$	Roseler et al. 1997; primiparous

\*DMI, kg/d; BW = body weight, kg; FCM = 4% fat corrected milk, kg/d; DIM = days in milk; WOL = week of lactation; MF = milk fat %; MP = milk protein %; BWC = body weight at parturition, kg; MPY = milk protein yield, kg/d.

\*\*Lag =  $1 - e^{-[(0.564 - 0.124 * pkmk) * (WOL + P)]}$ ; PKMK = month post calving when peak milk occurred (PKMK = 2); P = 2.36.

\*\*\*Lag =  $1 - e^{-[(0.564 - 0.124 * pkmk) * (WOL + P)]}$ ; PKMK = month post calving when peak milk occurred (PKMK = 3); P = 3.67.

## DMI EQUATION EVALUATIONS

Several evaluations of equations 1 through 9 have been published using independent data sets. The most commonly used evaluation criteria include:

- mean square predicted error (MSPE; kg of DMI<sup>2</sup>/d) =  $\sum(A-P)^2 / n$**   
where A = actual DMI (kg/d); P = predicted DMI (kg/d); n = the number of pairs of A and P values being compared;
- mean predicted error (MPE; kg/d) =  $\sqrt{\text{MSPE}}$**
- relative prediction error (RPE; MPE expressed as a % of observed mean DMI).**

Fuentes-Pila et al. (1996) evaluated several equations based on four independent data sets. The simplified equations [8 and 9] by Roseler et al. (1997b) were not separated by parity but instead by mo. of peak milk. The most accurate prediction of DMI for 2 of the 4 data sets and ranked 2<sup>nd</sup> for the other 2 data sets, was when the mo. of peak milk production occurred at 3 mo. (11.0, 16.2 and 9.5, 4.6 kg<sup>2</sup>/d, MSPE, respectively). The most accurate equation for the other 2 data sets was when peak milk production occurred at 2 mo. (9.1 and 4.6 kg<sup>2</sup>/d, MSPE, respectively) yet, for the other 2 data sets, the equation ranked 4<sup>th</sup> and 5<sup>th</sup> with MSPE of 25.1 and 28.0 kg<sup>2</sup>/d, respectively. Equations were also evaluated by parity and week of lactation (WOL) using four other independent data files (Fuentes-Pila et al., 1996). None of the equations evaluated, accurately predicted DMI consistently throughout the lactation. The equation by Roseler et al. (1997b) that used lag factors for peak milk at 3 mo. resulted in the most accurate prediction during early lactation (1 to 8 WOL). However, when week of lactation was greater than 8, DMI prediction accuracy decreased considerably.

Equation [1] developed by Kertz et al. (1991) that used data not split by parity and the cubic term for days in milk (DIM) was evaluated. Based on 3 data sets from Fuentes-Pila et al. (1996), the equation ranked 2<sup>nd</sup>, 4<sup>th</sup>, and 3<sup>rd</sup>, among other equations evaluated, with MSPE values of 11.2, 23.1, and 9.5 kg<sup>2</sup>/d, respectively. The equation predicted DMI more accurately during weeks 1 to 8 of lactation when compared with other equations but was considerably less accurate when week of lactation was greater than 8. Equation 1 was also evaluated by Roseler et al. (1997) using four data sets. When week of lactation was less than 26, equation [1] resulted in the lowest MSPE of the 7 equations evaluated for primiparous cows not treated with bST. For multiparous cows not treated with bST, equation [1] was the most accurate when WOL was less than 9 weeks with an MSPE of 12.1 kg<sup>2</sup>/d. The use of a third-degree polynomial for DIM appears to be responsible. Fuentes-Pila et al. stated that the model may be over-fitted resulting in reduced application for other data sets.

The equation [7] developed by Rayburn and Fox (1993) had the lowest accuracy for all 4 data sets (43.4, 40.0, 16.0, and 6.2 kg<sup>2</sup>/d, MSPE) as evaluated by Fuentes-Pila et al. (1996). Self-evaluation of equation [7] by Rayburn and Fox (1993) on an independent data set showed it consistently over-predicted DMI at low DMI (< 23 kg/d) and under-predicted at DMI >23 kg/d; R<sup>2</sup> = 0.43. When evaluated using the data sets by Roseler et al. (1997) for primiparous cows not treated with bST, Equation [7] resulted in MSPE ranging from 8.8 (kg<sup>2</sup>/d) for WOL < 10 to 32.3 (kg<sup>2</sup>/d) for WOL between 49 and 60. When ranked with the other 6 equations evaluated, Equation [7] ranked highest when WOL was between 25 and 36 (MSPE = 8.2 kg<sup>2</sup>/d). Results for multiparous cows not treated with bST were similar to those reported for primiparous cows.

Based on standards identified by Fuentes-Pila et al. (1996), all equations evaluated had poor accuracy and robustness (RPE consistently  $< \pm 20\%$ ) when evaluated using data from all stages of lactation.

Recent equation evaluators have not included Equation [4] and [5]. The NRC 2001 equation, published in January 2001, is too recent to have been evaluated and published.

### RECENT RESEARCH

Approximately 2,075 lactating Holstein cows are represented in a study that was conducted on three commercial dairy farms: one in southern Minnesota (Farm B) and two in western Wisconsin (Farm A and Farm C). Data was collected for 12 consecutive months at each location beginning in November 1999 for Farms A and C and January 2000 for Farm B.

#### Data Collection - All Farms

*Daily.* Group average milk production, DIM, lactation number, and cows per pen were electronically captured daily on each farm using Dairy COMP305<sup>®</sup> (Valley Agricultural Software, Tulare, CA). All daily farm production data was downloaded monthly to obtain complete 365 day records for each pen on each farm except on Farm C, the hard drive on the computer storing production data was erased resulting in a loss of data from April 25, 2000 to June 1, 2000. Bulk tank milk fat and milk true protein percent was determined daily on milk shipped from each farm.

All farms fed a total mixed ration. The average DM amount fed per head per day for each pen on all of the farms was recorded using EZ Feed software (Valley Agricultural Software, Tulare, CA). On Farm A, individual pen refusals were weighed almost daily whereas on Farms B and C weights of refusals were recorded as frequently as possible. Refusal DM amounts were calculated assuming the same DM concentration as the diet fed to the pen.

*Monthly.* Body weight was determined on 30 to 35% of the cows in each pen every month. No attempt was made to measure the same cows monthly, but a random sample of cows within the pen were measured either in headlocks using a heart girth tape (Farm A, Farm C) or weighed with an electronic scale exiting the parlor (Farm B). All cows were weighed at a similar time of day and month by location.

#### Farm A - Description

*Animals.* Eight hundred lactating Holstein cows were milked three times per day. Cows were grouped according to level of production and reproductive status within parity. Four early lactation pens out of the total of eight pens on the farm are represented in the data analysis (56% multiparous, 44% primiparous, of cows represented).

*Housing and Feeding.* Cows were housed in two six-row, curtain-sided freestall barns with headlocks. All diets were fed as a total mixed ration (TMR) once daily. The bunk management goal was to feed for an empty bunk at the same time every day and incorporate six scheduled feed push-up times per day. Diets averaged 53% forage, 47% concentrate on a DM basis with the forage portion consisting of 25% corn silage, 23% alfalfa haylage and 5% alfalfa hay.

### Farm B - Description

*Animals.* Six hundred Holstein cows were milked three times daily. Data was collected from four production groups: 1) Fresh cow group averaging <15 DIM, 2) First lactation cows, 3) Early lactation second and greater lactation cows 4) Mid-lactation to late lactation cows of mixed parity. Pens represented from Farm B averaged 144.3 DIM and produced 38.8 kg of milk/d.

*Housing and Feeding.* Cows were housed in three curtain-sided freestall barns. Barn 1 was a 3-row freestall barn with drive-by feeding along an open rail. Barn 2 was a four row with sand bedded freestalls and barn 3 was a 3-row freestall barn. Cows in both barn 2 and 3 were fed out of J-bunks. All cows were fed a TMR twice daily with six push-ups scheduled per day in barn 1. Diets averaged of 28% alfalfa haylage, 22% corn silage, 1% alfalfa hay and 49% concentrate, DM basis.

### Farm C - Description

*Animals.* Six hundred lactating Holstein cows were milked three times daily. Data was collected from eight lactation pens. Cows were divided into groups based on parity and reproductive status.

*Housing and Feeding.* Cows were housed in two 4-row free stall barns with headlocks along the feed bunk in each barn. All cows were fed a TMR once daily with feed pushups scheduled for several times a day. The feed bunk was managed for a target of 3% or less feed refusals with the daily TMR fed at a similar time each day. The diet DM consisted of corn silage (27%), alfalfa haylage (21%) and alfalfa hay (4%) and concentrate (48%).

## RESULTS AND DISCUSSION

The average number of cows, DMI, milk production, days in milk and lactation number for each of the three farms during the 12 month data collection period is in Table 3. The average farm DMI of lactating cows across the three farms was very similar during the data collection period averaging  $23 \pm 1$  kg/head/day. Farm A had the highest milk production per cow (39.9 kg/day), but the lowest DMI average (22.7 kg/day) of the three farms. This can be accounted for in that DMI was only measured on 4 pens on Farm A and these pens all contained cows in early lactation. All lactating cow pens on Farms B and C were measured and therefore, the data include cows in all stages of lactation. Farm A also had the lowest average lactation number and therefore, more first lactation animals were included in the data set as a percentage of total cows compared to Farms B and C.

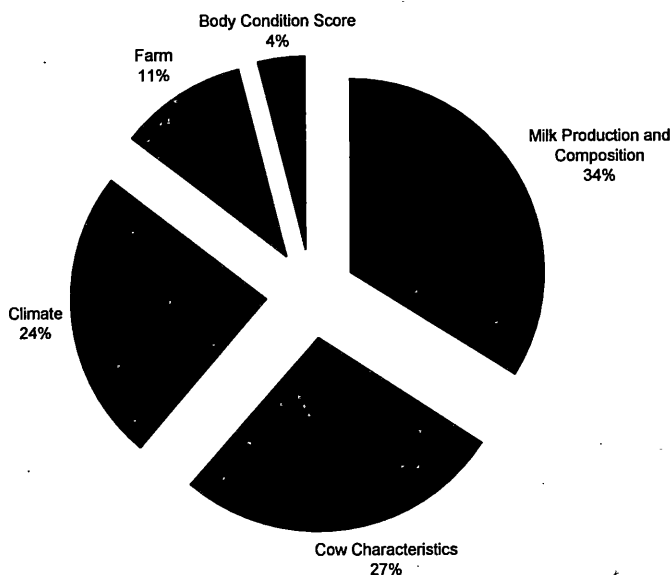
Table 3. Average farm data for the three commercial dairy farms from November 1999 to January 2001.

Farm	Number of Cows	DMI, kg/head/day	Milk, kg/head/day	Days in milk	Lactation number
A	353	$22.7 \pm 3.9^1$	$39.9 \pm 6.1$	$98 \pm 28$	2.1
B	1429	$23.3 \pm 4.9$	$38.8 \pm 9.1$	$145 \pm 106$	2.4
C	1002	$23.7 \pm 3.6$	$36.4 \pm 8.3$	$146 \pm 91$	2.3

<sup>1</sup> Standard deviation

Based on data collected daily from November 1999 to January 2001, principle component analysis was conducted to determine the primary variables that affect DMI. Figure 1 depicts the results. Milk production and composition and cow characteristics are the primary variables that determine DMI based on this data. Milk production was most closely related to DMI based on Pearson correlations (0.72). Cow characteristics included DIM, BW and lactation number. All three variables had similar correlations with actual DMI. Body condition score was not closely correlated with other cow characteristics and accounted for 4 percent of the variation in actual DMI. Climate accounted for 24 percent of the variation in DMI.

Figure 1. Variables that affect DMI in lactating dairy cows housed in freestall barns as determined using the principle component analysis of a large comprehensive database where 16 pens were the experimental unit and data was collected from November 1999 to January 2001.



Farm accounted for 11 percent of the variation in DMI. This is reflective of differences in management style between farms. Figure 2 and Figure 3 show the daily variation in DM fed for four pens on Farm B and four pens on Farm C. Farm B managed feed bunks that allowed cows to be fed when bunks were clean. In contrast, Farm C managed feed bunks so that all feed would be gone at a similar time each day. Based on these differences, Farm B contains more daily variation in DM fed than Farm C as depicted in Figure 2 and 3.

## EQUATION EVALUATION

Equations [2] through [9] were evaluated based on the data collected. Relative prediction error (RPE; MPE/mean DMI for the corresponding data file) was used to determine when the prediction accuracy could be considered acceptable or not acceptable (Fuentes-Pila, 1996). An RPE lower than 10% indicated satisfactory prediction of DMI, RPE between 10 and 20% was a relatively good or acceptable prediction and RPE greater than 20% indicated an unsatisfactory prediction. Each equation was evaluated using data from multiparous and primiparous pens combined. All data used to evaluate equations was from the single day that BW and BCS were measured on the farms. Results should reflect the ability of each equation to accurately predict DMI based on data that may be retrieved by a nutritionist on their visit to a farm. Each equation was evaluated using individual farm and overall data.

Accuracy of the eight prediction equations by farm and overall is shown in Table 4. The best three overall equations were the NRC 2001 equation [5] and the two equations published by May 1994 [4], [3], (RPE < 10%). The MSPE of equations [5], [4] and [3], based on overall data, was still higher than that reported by the NRC 2001 (3.31 kg<sup>2</sup>/d, MSPE) when data from individual cows was used.

Three equations met our criteria for satisfactory prediction of DMI when using the overall data set (Equation [3], [4], and [5]). However, all eight equations evaluated resulted in an acceptable prediction of DMI based on our criteria of RPE < 20% when accurate data was collected from a farm on a specific day.

Table 4. The mean square predicted error (MSPE) for eight published equations based on average pen data collected once per month from three commercial dairy farms<sup>1</sup>.

Equation	Farm A		Farm B		Farm C		Overall	
	MSPE <sup>2</sup>	RPE <sup>3</sup>	MSPE	RPE	MSPE	RPE	MSPE	RPE
N	45 (45) <sup>3</sup>		48 (32)		79 (49)		172 (126)	
[1]	16.78	18.06	20.24	17.89	7.93	12.05	14.22	15.91
[2]	7.33	11.94	9.12	12.03	3.79	8.33	6.41	10.68
[3]	6.10	10.88	8.83	12.75	1.43	5.08	4.72	9.31
[4]	5.47	10.30	8.26	12.32	1.61	5.38	4.47	9.06
[5]	4.90	9.74	8.44	12.48	1.76	5.63	4.45	9.06
[6]	9.94	13.87	11.89	14.81	3.19	7.58	7.38	11.67
[7]	11.92	15.29	10.71	14.03	2.23	6.31	7.13	11.46
[8]	6.64	11.36	10.82	14.12	2.07	6.10	5.71	10.26

<sup>1</sup>N = 45 (45), 48 (32), 79 (49) and 172 (126) observations for Farm A, Farm B, Farm C, and Overall, respectively. Values in parenthesis are the number of observations included in the analysis of equation [1] and [2] (Table 1) when DIM < 150.

<sup>2</sup>MSPE =  $\Sigma(A-P)^2/n$ ; where A=actual DMI (kg/d), P=predicted DMI (kg/d), n=number of pairs of A and P; mean square predicted error, kilograms squared per day.

<sup>3</sup>RPE =  $(\sqrt{\text{MSPE}})/\text{actual average DMI}$  for respective population; relative predicted error, %. For equation [1] and [2], actual average DMI used was from pens < 150 DIM.

## CONCLUSION

The eight previously published equations resulted in an acceptable prediction of DMI (RPE < 20%). Equations published by May (1994) and NRC 2001 resulted in the most accurate prediction of actual DMI based on MSPE. Determination of actual BW, DIM and 4% FCM for a pen of lactating Holstein cows on a specific day will result in a prediction of DMI that is similar to actual based on data collected across three commercial dairy farms. Several management factors may play a role in the accuracy of prediction equations across farms. Therefore, accurate measurement of DMI on each farm will always be the best measure of DMI to maximize profitability.

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# EVALUATION OF THE 2001 DAIRY NRC ENERGY AND PROTEIN REQUIREMENTS

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## INTRODUCTION

The 7<sup>th</sup> Edition of the Nutrient Requirements of Dairy Cattle (NRC-2001) was released in January 2001. Previous editions of the Dairy NRC contained nutrient requirements of animals along with projected dietary concentrations needed to meet animal requirements. However, the dietary nutrient requirements were static and did not directly account for animal or feedstuff variations that could affect the requirement or supply of nutrients. The NRC-2001 relies heavily on a computer model to dynamically predict dietary nutrient requirements based on animal type, production, environment, and feedstuffs being fed. Thus, the dietary nutrient requirements in the NRC 2001 consider feedstuff digestion dynamics as well as the actual nutrient requirements for maintenance, growth, lactation, reproductive status and activity of the animal.

The purpose of this paper is to review the energy and protein systems in NRC-2001 with emphasis on the lactating cow. In evaluating the protein and energy requirements from the NRC-2001, both animal requirements and supply of nutrients must be considered as well as the interaction between them. In any diet evaluation, accurate input information is required. However, in a dynamic model like the NRC-2001, the users responsibility for accurately defining animals, diets and management conditions is increased. This paper will help users interpret protein and energy requirements from the model and identify areas for input error.

## DRY MATTER INTAKE

The NRC-2001 contains a DMI prediction equation for lactating cows. The equation for lactating cows was developed from over 17,000 cow weeks of DMI. The database included approximately 1/3 first lactation and 2/3 second or later lactation cows, and represented a wide array of dietary ingredients and feeding management programs. The equation developed is a combined equation of two published equations (Rayburn and Fox, 1993; Roseler et al., 1997). The following equation for predicting DMI of lactating cows is universal in that it is applicable during all stages of lactation, and to cows in first lactation and greater:

$$\text{DMI (kg/d)} = (0.372 \times 4\% \text{ FCM} + 0.0968 \times \text{BW}^{.75}) \times (1 - e^{(-0.192 \times (\text{WOL} + 3.67))})$$

4% FCM = 4% fat corrected milk

BW = body weight (kg)

e = 2.71828

WOL = week of lactation

The term  $(1 - e^{(-0.192 \times (\text{WOL} + 3.67))})$  adjusts for the lowered DMI during early lactation. Differences in DMI between first and second or later lactation cows will be accurately differentiated with the use of correct BW and 4% FCM. A difference of 100 kg in BW changes DMI by 1.5 kg/day. It

is important for users of the equation to enter accurate 4% FCM, BW and WOL for the group or herd of cows being evaluated.

The DMI equation for lactating cows provides a good estimate of DMI, but it does not replace the need for or use of actual information. DMI is a critical component in the model's derivation of animal requirements and the evaluation of diets to meet requirements. Discount factors for energy values and rumen undegradable protein (RUP) are two examples where DMI significantly affects the values computed by the model. Over-estimating DMI results in a lower energy concentration and a higher RUP content of the diet than actual; vice versa, underestimating DMI inflates energy concentration in the diet and decreases RUP content.

The DMI information used to develop the predictive equation shows a very different pattern of DMI for first lactation cows and cows of second or greater lactation during 48 weeks of lactation (Figure 1). First lactation cows have a slow, steady rise in DMI during early lactation reaching a plateau at about 16 weeks and remaining there for the duration of the lactation. In contrast, older cows increase DMI rapidly the first few weeks of lactation peaking at 5 to 6 weeks of lactation and then slowly decrease as lactation progresses.

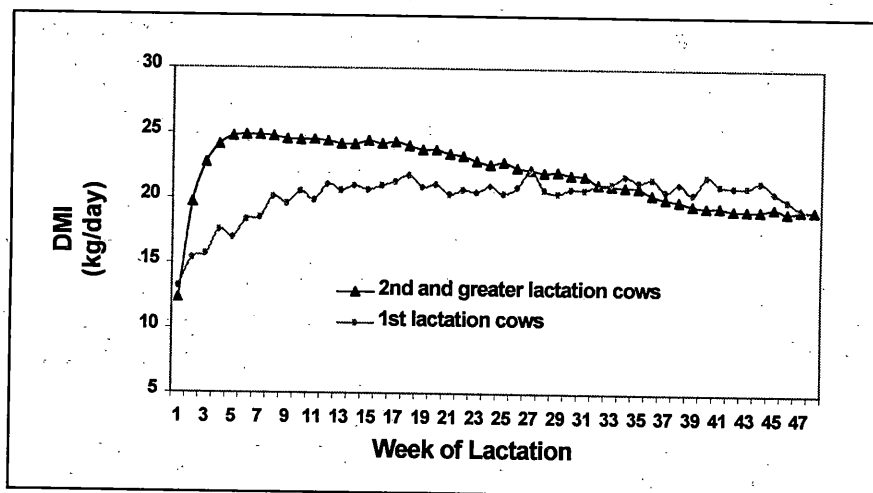


Figure 1. DMI of first lactation cows and second or greater lactation cows during the first 48 weeks of lactation.

## ENERGY

Feed and diet energy. The approach used in the NRC-2001 to calculate energy values of feeds and diets is substantially different than that used in previous editions of the NRC. In older editions, digestible energy (DE), metabolizable (ME) and net energy of lactation ( $NE_L$ ) value of most feeds was calculated from an experimentally determined TDN value. Limitations to this method were:

- TDN values for most feeds were determined many years ago.

- For several feeds, the TDN value cannot be determined directly as they cannot be the sole ingredient in a diet. Therefore, inaccuracies in calculating the TDN of a single feed in a diet of mixed feeds can occur because of associative effects.
- Nutrient composition of feeds has changed over the years, but TDN value did not.
- Energy values for feeds were computed at a constant intake of 3 times maintenance. This single intake level is not correct for many cows and herds today. Also, the depressing effect increased DMI has on energy content or digestibility of the diet was not accounted for by using a single DMI level.

In NRC-2001, the energy value of feeds and diets are calculated from nutrient composition information. The equations for calculating the DE of feeds or diet at maintenance are shown below. The approach is to multiply each nutrient fraction by its appropriate heat of combustion to determine the truly digestible (td) nutrient component. The td components are then summed and a metabolic fecal fraction (0.3) is subtracted to obtain the DE at maintenance.

Feed fraction - truly digestible (td)	Equation
1a Crude protein forages (td CP <sub>f</sub> )	= [(CP x exp <sup>(-0.012 x ADICP/CP)</sup> )] x (5.6 <sup>**</sup> /100)
1b CP concentrates (td CP <sub>c</sub> )	= [(1 - (.04 x ADICP/CP)) x CP] x (5.6 <sup>**</sup> /100)
2 Nonfiber carbohydrates (td NFC)	= [(98 x (100 - [(NDF - NDICP) + CP + EE + Ash])) x PAF] x (4.2 <sup>**</sup> /100)
3a Fatty acids (td FA) or	= FA x (9.4 <sup>**</sup> /100)
3b Ether extract (td EE)	= (EE - 1) x (9.4 <sup>**</sup> /100)
4 Neutral detergent fiber (td NDF)	= [0.75 x ((NDF - NDICP) - Lignin) x (1 - (Lignin/(NDF - NDICP)) <sup>0.667</sup> )] x (4.2 <sup>**</sup> /100)
DE, Mcal/kg	= [1a or 1b] + [2] + [3a or 3b] + [4] - 0.3

\* All composition data is expressed as a percent of the dry matter. ADICP = acid detergent insoluble nitrogen x 6.25; NDICP = neutral detergent insoluble nitrogen x 6.25; PAF = processing adjustment factor.

\*\* Heat of combustion (Mcal/kg) values for various nutrients.

Because DE at maintenance is not representative of the energy value of a feed or diet at production intake levels, a discount factor based on DMI and TDN content of the diet at maintenance (TDN<sub>IX</sub>), was developed to correct for decreased digestibility as DM intake increased. An intake corrected DE (discounted DE) is then used to calculate ME and finally NE<sub>L</sub>. This approach means the energy value of feeds and diets will not be the same at different intakes.

The following equations are used to convert DE at maintenance to production levels of DE<sub>p</sub>, ME<sub>p</sub> and NE<sub>Lp</sub>.

$$DE_p, \text{Mcal/kg} = DE \times \text{discount factor}$$

$$ME_p, \text{Mcal/kg} = (1.01 \times DE_p - 0.45) + (0.0046 \times (EE - 3))$$

$$NE_{Lp}, \text{Mcal/kg} = (.703 \times ME_p - 0.19) + \{[(0.097 \times ME_p + 0.19)/0.97] \times (EE - 3)\}$$

Energy discount factor. The digestibility or energy concentration of diets decreases with increasing DMI. The standard discount, applied to energy values in NRC-1989 was a 4% reduction from maintenance energy value per multiple of DMI above maintenance. Almost all feed tables and diets in NRC-1989 used energy values at 3 times (3X) maintenance DMI for an 8% discount in energy value from maintenance. In NRC-2001, a variable discount is applied to the DE of diet based on TDN<sub>1X</sub> and DMI. The base equation for calculating TDN<sub>1X</sub> is:

$$\text{TDN}_{1X}, (\%) = (\text{tdNFC} + \text{tdCP} + \text{tdEE} + \text{tdNDF}) - 7$$

tdNFC = truly digestible nonfiber carbohydrates  
 tdCP = truly digestible crude protein  
 tdEE = truly digestible ether extract  
 tdNDF = truly digestible neutral detergent fiber  
 7 = metabolic fecal TDN value

Equations for calculating the truly digestible nutrient fractions can be found in Chapter 2 of the NRC-2001 publication. Adjustments to the above TDN<sub>1X</sub> equation are made for animal protein meals because of no structural carbohydrates, and for fat supplements.

$$\text{Discount factor} = (\text{TDN}_{1X} - [((0.18 \times \text{TDN}_{1X}) - 10.3) \times \text{Intake}] / \text{TDN}_{1X}$$

For example, a cow eating 21 kg of DMI per day with a maintenance DMI of 7 kg is eating at 3X maintenance (21 kg/7 kg). Intake above maintenance is 2 (3X - 1X for maintenance). If maintenance TDN (TDN<sub>1X</sub>) is 75%, a discount of 0.915 is applied to maintenance DE to calculate a production DE<sub>p</sub>. No discount is applied to diets below 60% TDN<sub>1X</sub>.

Processing adjustment factor (PAF). Because starch availability of a feed can be affected by physical or chemical processing, a PAF factor was developed to account for the differences in starch digestibility and, hence, energy value of the feed. The PAF is an empirical factor based on dividing in vivo starch digestibility of the feed by 0.9. Ground corn is generally accepted as the standard and was found to have an in vivo starch digestibility of about 90%; thus, the PAF of ground corn is 1. For cracked dry corn where starch would be less available for digestion, the PAF is 0.95 and for steamed flaked corn with higher starch digestion than ground corn, the PAF is 1.04. The PAF adjustment is applied only to the nonfiber carbohydrate (NFC) fraction of the truly digestible NFC (tdNFC) equation.

Carbohydrates. The NRC-2001 acknowledges two equations for calculating NFC. The equation used in energy calculations and the most correct equation for estimating NFC because it does not double count the CP in the NDF fraction is:

$$(1) \text{ NFC, \%} = 100 - (\text{CP, \%} + \text{Fat, \%} + \text{Ash, \%} + \text{NDF, \%} + \text{NDFICP, \%})$$

The NFC equation used in providing dietary NFC recommendations (Table 1) is:

$$(2) \text{ NFC, \%} = 100 - (\text{CP, \%} + \text{Fat, \%} + \text{Ash, \%} + \text{NDF, \%})$$

The NFC values from equation 1 will generally be 2 to 4% higher than from equation 2. Thus, if equation 1 is used to calculate NFC values, the guidelines in Table 1 should be adjusted to reflect the difference.

Recommendations for fiber and nonfiber carbohydrates (NFC) in lactating cow diets are shown in Table 1. The total NDF, NDF from forage and acid detergent fiber (ADF) recommendations, are minimums; whereas, NFC recommendations are maximums. An important relationship among the values in Table 1 is that as forage NDF decreases, total NDF must increase and NFC should decrease. This will reduce the risk of acidosis when low forage diets are fed.

Table 1. Recommended total NDF, forage NDF, ADF, and NFC concentrations in the diets of lactating cows fed total mixed rations<sup>1</sup>

Minimum % of diet DM			Maximum % of diet DM
Forage NDF	Total NDF	ADF	NFC <sup>2</sup>
19	25	17	44
18	27	18	42
17	29	19	40
16	31	20	38
15	33	21	36

<sup>1</sup> Assumes forage particle size is adequate and ground dry corn is starch source.

<sup>2</sup> NFC = 100 – (NDF + CP + Fat + EE). All analyses are % on a DM basis.

Forage particle size and/or effective fiber recommendations are commonly given in the field, but the lack of standard validated measures and published information relating these measures to requirements precluded NRC-2001 from establishing specific recommendations for these parameters in diets. Several research studies have shown that a minimum forage particle length of 3 mm is needed to maintain good rumen pH and adequate rumination activity, and prevent depressions in milk fat percentage (Allen, 1997; Beauchemin et al., 1994; Grant et al., 1990a,b). The Penn State shaker box for particle sizing forages is an excellent field tool, but quantifying screen particle size to chewing activity, rumen health and milk fat percentage is needed.

Feed energy values. The NE<sub>L</sub> value of feeds in NRC-2001 averages 2% lower at 3X than those found in the previous edition. Feeds decreasing most in energy value were forages and, particularly, low quality forages. High protein feeds generally increased in energy value while most grains (starch sources) have a similar energy value to NRC-1989. The NE<sub>L</sub> value change from NRC-1989 to NRC-2001 for some common feed ingredients is shown in Table 2.

Energy requirements. The energy requirement for maintenance of lactating and dry cows is: maintenance NE<sub>L</sub> (Mcal/day) = 0.08 x BW<sup>0.75</sup>. In NRC-2001, milk protein and milk lactose components are considered in determining lactation requirements in addition to fat, which was only considered in NRC-1989. For most Holsteins, there is no noticeable difference in lactation requirements, but requirements have increased slightly for high component cows.

The NE<sub>L</sub> required for maintenance includes a 10% increase for activity. This should be satisfactory for most non-grazing tie stall housed cows. However, for grazing cows or cows in free stalls or dry lot facilities that are walking considerable distances for feed and/or to the milking parlor, additional energy above maintenance and lactation will be required. In NE<sub>L</sub>

units, the energy required for activity is set at 0.00045 Mcal/kg BW for every kilometer walked. A 600-kg cow that walks 2 kilometers per day needs an additional 0.54 Mcal of energy per day or about a 5.5% increase in maintenance requirement.

The pregnancy requirement was fixed at 30% of maintenance in the NRC-1989. In NRC-2001, the energy requirement for gestation increases with gestation length. Below 190 days of gestation, no additional energy above maintenance is needed for pregnancy. Between 190 and 279 days of gestation, pregnancy requirements of the average Holstein cow increase from 2.5 to 3.7 Mcal/day, respectively. No increase in pregnancy requirement occurs beyond 279 days.

Table 2. NE<sub>L</sub> values at 3X (DM basis) of some common feed ingredients and change from 1989 to 2001 Dairy NRC.

Feed	1989 edition	2001 edition	% of 1989
Alfalfa hay <40% NDF	0.68	0.62	91.2
Alfalfa hay >46% NDF	0.59	0.51	86.4
Corn silage - average	0.73	0.71	97.3
Barley	0.88	0.84	95.5
Corn grain, ground	0.89	0.91	102.2
Corn, flaked	0.93	0.95	102.2
Corn gluten feed	0.87	0.78	89.7
Hominy	0.91	0.92	101.1
Bakery byproduct	0.94	1.00	106.4
Brewers grains, wet	0.68	0.78	114.7
Cottonseed, lint	1.01	0.88	87.1
Molasses, beet	0.78	0.81	103.8
Wheat midds	0.71	0.76	107.0
Blood meal, ring	0.68	1.06	155.9
Distillers grain/solubles	0.93	0.89	95.7
Soybean meal-44	0.88	0.96	109.1
Soybeans, roasted	0.99	1.23	124.2
Fats			
Calcium soaps		2.28	
Tallow			
Hydrolyzed	2.65	2.45	92.5
Partially hydrogenated		1.35	
Vegetable oils	2.65	2.56	96.6

## PROTEIN

Dairy animals require amino acids and not protein directly. The collective amino acid requirement is defined as metabolizable protein (MP), which is the total amino acids absorbed from the small intestine. Sources of MP are rumen-undegradable protein (RUP) from feeds, ruminally synthesized microbial crude protein (MCP), and endogenous protein sources (Figure 2).

Microbial crude protein (MCP) yield is estimated at 130g/kg of production or discounted TDN (TDN<sub>p</sub>) when rumen-degradable protein (RDP) (kg/day) exceeds 1.18 x the TDN<sub>p</sub>-predicted MCP yield. This ensures there is adequate RDP in the diet for MCP synthesis. When RDP (kg/day) is less than 1.18 x TDN<sub>p</sub>, then MCP yield is reduced to 0.85 x RDP (kg/day). Predicted MCP predicted in the NRC-2001 is 11 to 27% lower than in the NRC-1989. The true protein content of MCP is 80% of the CP and the digestibility of MCP in the intestine is estimated to be 80%; therefore, MP from MCP is 64% of the total MCP yield. Both RUP and endogenous proteins are considered to be 100% true protein.

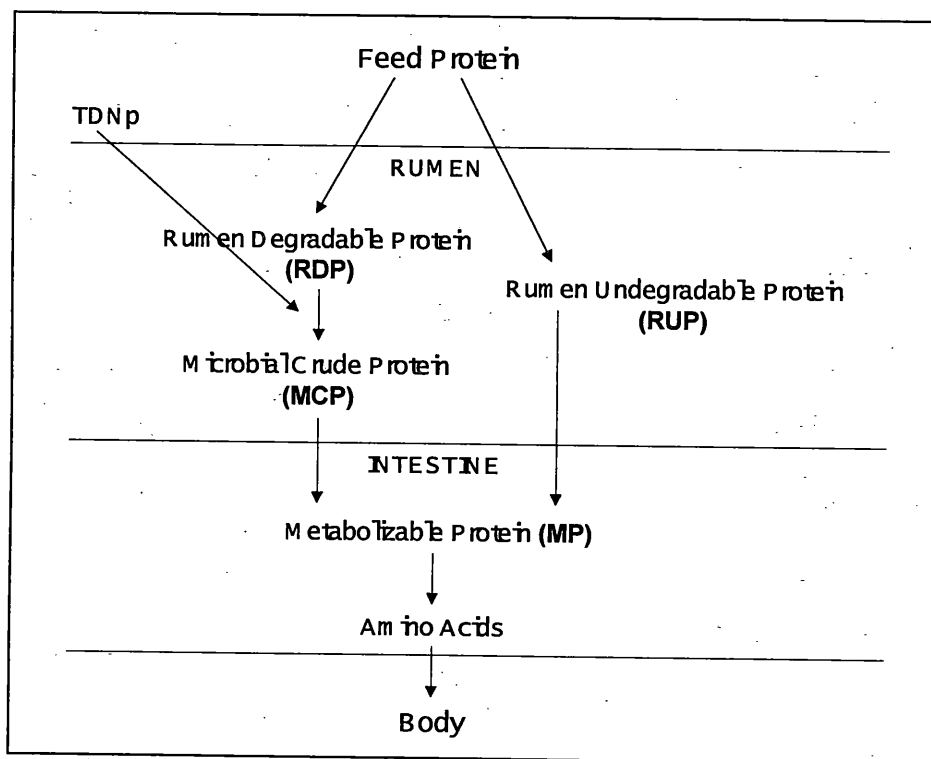


Figure 2. Origin of MP from feed protein fractions and MCP.

Feed protein. The CP in feeds is divided into three fractions (A, B and C). Fraction A is the percentage of CP that is nonprotein nitrogen (NPN) and true protein immediately soluble in rumen fluid. Fraction C is the percentage of CP that remains in an in situ bag after a defined end-point time. Fraction B is the remainder of the CP (Total CP – (Fractions A + C)) and is considered to be degradable in the rumen over a wide time span.

The RDP value for a feed is fraction A plus that portion of fraction B that disappears from an in situ bag within 48 hours (general feeds) or 72 hours (forages). The RUP is fraction C plus the portion of fraction B remaining in the bag after the defined rumen fermentation time period. In NRC-2001, in situ analyses were used to establish the RDP and RUP fractions of feeds and the rate of ruminal degradation ( $k_d$ ) and feed passage ( $k_p$ ) was used in their derivation. Equations for calculating RDP and RUP are:

$$\text{RDP} = \text{Fraction A} + \text{Fraction B} \left[ \frac{k_d}{k_d + k_p} \right]$$

$$\text{RUP} = \text{Fraction B} \left[ \frac{k_d}{k_d + k_p} \right] + \text{Fraction C}$$

Fraction A, B and C are % of CP  
 $k_d$  = rate of degradation of Fraction B in the rumen, %/hour  
 $k_p$  = rate of passage of feedstuff from the rumen, %/hour

The passage rate of feed from the rumen is a function of DMI and for forages, also the moisture content. For all feeds, as total DMI increases the rate of feed passage from the rumen ( $k_p$ ) increases. Passage from the rumen is faster for wet forages (silages, fresh forage) than for dry forages. Passage of dry forages also is reduced as NDF content increases and the percent concentrate in the diet increases. The effect of increasing DMI on RUP value of feeds and the diet is shown in Table 3.

Table 3. Rumen undegradable protein (RUP) values of feeds in a diet as DM intake increases from 18.3 to 27.2 kg/day or 2.9 to 4.4% of BW.

Feed	% of DM	2.9% of BW	4.4% of BW
		RUP as % of CP	
Alfalfa hay	7.9	15.7	16.9
Alfalfa haylage – 40% DM	27.8	17.2	18.2
Corn silage – 35% DM	27.8	34.3	35.6
Corn grain	19.8	44.2	49.0
Distillers dried grain/solubles	7.1	48.3	52.2
Soybean meal-44%	7.1	38.9	44.7
Blood meal, ring dried	.9	75.6	78.4
Total diet		31.9	34.5

The limitation to the NRC-2001 protein system is the lack of analytical methods to measure fractions A, B and C and the difficulty of accurately knowing  $k_d$  and  $k_p$  in most practical situations. In situ analysis can divide feed protein into fractions A, B and C, but the procedure is not routine, not offered by many commercial laboratories and is expensive. A practical but not totally accurate approach to estimate fractions A, B and C for fermented forages is to use soluble protein and ADICP. Soluble protein (% of total CP) would represent fraction A and ADICP (% of CP) could be considered fraction C and B would be  $100 - (A+C)$ .

**MP requirements.** A factorial approach was used for predicting MP requirements for maintenance, growth, pregnancy and lactation.

Maintenance MP requirement includes requirements for scurf, urinary excretion, metabolic fecal protein and endogenous protein.

$$\text{Maintenance MP (g/day)} = (0.3 \times (\text{BW} - \text{CW})^{0.60}) + (4.1 \times (\text{BW} - \text{CW})^{0.50}) + [(\text{DMI} \times 30) - 0.5 \times ((\text{bacterial MP}/0.80) - \text{bacterial MP})] + [(0.4 \times (11.8 \times \text{DMI}))/0.67]$$

BW is body weight (kg); CW is conceptus weight (kg)

Growth MP requirements are based on live weight gain and the NE content of live weight gain. Two equations are used depending on equivalent shrunk BW (EQSBW), one for less than or equal to 478 kg  $\{[\text{WG} \times (268 - (29.4 \times (\text{RE}/\text{ADG})))] / [(83.4 - (0.114 \times \text{EQSBW}))/100]$  and one

for greater than 478 kg  $\{[WG \times (268 - (29.4 \times (RE/ADG)))]/0.28908\}$ . Where WG is weight gain, RE is retained energy, and ADG is average daily gain (all values are in kg). The difference in the two equations is the denominator. For EQSBW less than 478 kg, the efficiency of MP use for growth is variable; whereas, above 478 kg, the efficiency is assumed to be constant at 28.9%.

Pregnancy MP requirements are calculated for days 190 to 279 of gestation. The equation for calculating MP for pregnancy is  $\{[(0.69 \times \text{days pregnant}) - 69.2] \times (\text{calf birth weight (kg)/45})\}/0.33$ . The efficiency of conversion of MP to conceptus protein is assumed to be 33%.

The MP requirement for lactation is based on milk protein yield and an efficiency of converting MP to milk protein of 67%. The MP lactation equation is milk protein yield/0.67.

Dietary protein requirements. Equations for estimating dietary RDP and RUP requirements are:

$$\text{RDP, \% of diet DM} = ((0.15294 \times \text{TDN}_p \text{ (g/day)})/\text{DMI}) \times 100$$

$$\text{RUP, \% of diet DM} = \left[ \frac{\text{MP required} - \text{MP from bacterial and endogenous sources}}{\frac{\text{RUP Digestibility}}{\text{DMI}}} \right] \times 100$$

The CP content of the diet DM is the sum of the RDP% and RUP% of diet DM.

Amino acids. The NRC-2001 computer model predicts concentrations of essential amino acids (EAA) in MP. Knowledge was too limited for the committee to establish actual amino acid requirements of lactating cows and to construction a model to quantify amino acid requirements. However, the optimum use of MP for maintenance and lactation is estimated to be when lysine and methionine are 7.2% and 2.4% of MP, respectively and in a 3 to 1 ratio.

## DIET EVALUATIONS – LACTATING COW

Diet information was collected from two Minnesota dairy farms and entered into the NRC-2001 computer model for evaluation. Diet information and the energy and protein output from the model are in Table 4. The following is presented to show some of the information available from the model and how to interpret the information in evaluating diets.

### *Animal parameters used:*

	Farm A	Farm B
Body weight, lb*	1400	1400
Milk production, lb/day	75.0	106.0
Days in milk	150	180
Milk fat, %	4.0	3.5
Milk protein, %	3.1	3.0
Age, months*	48	43
Days pregnant*	150	60
Body condition score*	3.0	3.0
Lactation number*	2.0	2.0

\* Indicates data was estimated and not measured or quantitatively obtained.

## ***Feed information***

Farm A – Ration and production information was obtained from the farm. A basic forage test had been conducted on the haylage and corn silage. No other nutrient information on ingredients was available.

Farm B – Ration and production information was obtained from the farm. The forage tests on haylage and corn silage contained more detailed information such as NDICP, soluble protein, ADICP, fat and ash. No other nutrient information on ingredients was available.

## ***Diet Comments***

### **Farm A**

**Input information:** Most of the animal and feed information needed for input into the model was estimated and not quantitatively measured. Most animal information such as BW, BCS, days pregnant, age, and lactation number are estimated for use in ration formulation. If accurate information on energy and protein feeding are to be obtained, more effort into quantifying as much animal information as possible is needed. The basic forage test of DM, CP, ADF and NDF is insufficient for use in model evaluations. More comprehensive nutrient information on protein and carbohydrate fractions in forages and feeds is needed. The nutrient content of feeds in the feed libraries of the NRC-2001 model and of the computer program the nutritionist uses to formulate diets must be relatively similar. This problem is illustrated in the CP % of the diet formulated by the nutritionist being 2% units lower than same ingredients and amounts analyzed through NRC-2001 model. A discrepancy in nutrient content like this makes interpretation of any results questionable and/or questions the accuracy of the diet being fed on the farm.

**DMI:** Model prediction of DMI was similar to the reported actual on farm average. The actual DMI was used in the diet analysis. Notice the DMI over maintenance is 3.6X in this average Minnesota dairy herd and not the standard 3X used in the previous NRC (1989).

**Milk production:** The  $NE_L$  and the MP allowable milk, 79.0 and 75.2 lb/day, respectively are very similar to actual milk at 75.0 lb/day. However, the projected milk productions are based on a 19.2% CP and not the reported 17.1% CP diet. If the 17.1% CP is correct, MP allowable milk projections would decrease to about 71 lb/day.

**$NE_L$ :** The difference between the  $NE_L$  concentration in the ration and the NRC-2001 value illustrates the effects of changes in derivation of energy values and the variable discounting of energy concentration in the diet based on DMI rather than a set 3X. Based on NRC-1989, the .75  $NE_L$  diet would be acceptable for 75 lb of milk. A .70  $NE_L$  value for the same diet using the NRC-2001 supports the same milk production and allows for a projected BW gain of .6 lb/day. Users of NRC-2001 are reminded that amounts of nutrients (concentration times DMI) are required for production and not just concentration in the diet.

**Protein fractions:** Information on most of the protein fractions in Table 4 was unavailable from NRC-1989. MCP yield was estimated from  $NE_L$  in the diet, but not corrected for  $NE_L$  coming from fat; thus, an over-estimation of MCP yield often occurred. Information from

the NRC-2001 model indicates RDP is being supplied in excess of 2 lb/day at the 19.2% CP concentration. Assuming the same balance of RDP and RUP in the diet, the 17.1% CP diet supplies 5.6 lb/day of RDP for an overage of about .6 lb/day.

Differences in RDP and RUP (% of CP) between the actual and NRC-2001 diets are from newer values for these two protein fractions for feeds and the use of the discount factor associated with DMI being applied to change the RDP of feeds in the NRC-2001 model. In NRC-1989, the RDP and RUP fraction in feeds were static and not adjusted for DMI whereas in NRC-2001, the RDP of feeds decrease as residence time in the rumen decreases with increasing DMI. The sum of RDP and RUP (when expressed as % of diet DM) = %CP in the diet DM. In most lactating cow diets, the RDP should be greater than 11% of the DM to avoid limiting microbial growth and optimizing rumen digestion of feedstuffs.

**Amino Acids:** The target of 7.2% lysine and 2.4% methionine in MP was not achieved with the current feeding program. The ratio in the diet is 3.6 to 1 rather than desired 3 to 1. Strategies to correct both the shortage of lysine and methionine as well as the ratio include changes in protein supplementation and increasing microbial protein production. If RDP is high as in this situation, RUP sources can replace some of the RDP in the diet. If RDP is marginal, adding RUP sources will decrease microbial protein production and probably lower both lysine in MP and total MP.

### Farm B

Many of the comments about Farm A diet are applicable to Farm B. Only specific points relative to the model output about Farm B diet will be discussed below.

**DMI:** Again, the model is relatively accurate in predicting the amount of feed fed. The DMI predicted by the model is true intake and not what is fed. On most farms, the fed amount is probably 5 to 10% above the actual DM consumed. The energy and protein discount applied to Farm B diet is 5X over maintenance or 16.8%.

**Milk production:** The  $NE_L$  allowable milk is 7 lb/day greater than the actual and thus, energy because of the high DMI is not a limiting nutrient in this diet. Projected milk from MP is 8 lb/day less than actual and therefore, RUP and/or some essential amino acids are the limiting nutrient in this diet.

**Protein fractions:** The CP content of the actual and NRC-2001 model diets is very close considering the number of ingredients and probable difference in feed library nutrient content. The amount of RDP is in excess (1.7 lb/day). The RDP as a % of the DM is 12.7, but decreasing RDP to the amount required would decrease the RDP concentration in the diet to 10% of the DM and considerably lower than the desired minimum of 11% of the DM. The contribution of both bacterial and diet RUP to MP increase with increasing DMI.

**Amino Acids:** Both total lysine and methionine and the ratio between them is lower than what is considered to be optimum.

Table 4.

## Diets

Feed	Diets	
	Farm A	Farm B
	----- lb/head/day as fed -----	
Corn silage	27.5	45
Haylage	43	49
Corn grain source	8.6	12.5
Corn gluten feed	5.4	4.5
Cottonseed, fuzzy		3.5
Soybean meal-44%		1.1
Soybeans/Protected SBM	4.3	1.5
Urea		0.1
Linseed meal	0.8	0.5
Corn distillers/solubles		0.8
Animal protein sources	.9	0.5
Corn gluten meal	0.3	0.5
Midds		0.5
Cereal byproduct		0.5
Mineral/vitamin/other	2.0	3.4
Total	92.8	123.9

## DIET ANALYSIS

	Farm A		Farm B	
	Ration Spec	NRC-2001	Ration Spec	NRC-2001
DMI, lb/day	52.2*	53.8**	62.1*	62.6**
Intake over maintenance	3.6X		5.0X	
Milk, lb/day	75		106	
Allowable milk, lb/day				
NE <sub>L</sub>		79		113
MP		75.4		98
		ENERGY		
Diet NE <sub>L</sub> , Mcal/lb	.75	.70	.76	.73
NFC (NSC), % of DM	(32.6)	35.5	38.4	39.5
NDF, % of DM	34.2	34.4	29.3	29.7
		PROTEIN		
CP, % of DM	17.1	19.2	18.8	18.4
RDP, % of CP	62.4	70.3	67.0	69.0
RDP required, lb		5.0		6.2
RDP supplied, lb		7.0		7.9
RUP, % of CP	37.6	29.7	31.4	31.0
RUP required, lb		2.9		4.0
RUP supplied, lb		2.9		3.6
MCP yield, lb		4.2		5.2
		AMINO ACIDS		
Total essential, lb/day		2.9		3.6
Lysine, % of MP		6.4		6.3
Methionine, % of MP		1.8		1.9

\* Actual DMI reported from farm and used in NRC model evaluation.

\*\* DMI predicted by NRC-2001 model.

## SUMMARY

The model in 7th Edition of Nutrient Requirements of Dairy Cattle predicts nutrient requirements of dairy animals and evaluates diets for their competency to meet the requirements. Users of the model will find necessary dietary requirements to meet static animal nutrient requirements changing as feeds in the diet change and as DMI changes. This is a powerful unique feature that provides nutritionists with a much greater explanation about animal performance related to the diet than previously was attainable from generalized nutrient requirement and feed composition tables. However, as the sophistication of tools to solve problems increases, so does the need to learn new concepts and approaches to solving the problem. Nutritionists are encouraged to learn and use the new terms, concepts and approaches to feeding dairy cattle described in the 7th Edition of Nutrient Requirements of Dairy Cattle.

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# EVALUATING THE ENERGY CONTENT OF FAT SUPPLEMENTS IN RUMINANT DIETS

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## INTRODUCTION

Until recently, the energy content of fat was considered to be similar among various sources. For example, in the 1989 NRC for dairy cattle, there were three fat sources listed (hydrolyzed animal fat, lard, and vegetable oil) and the same energy value was assigned to each. Additionally, there was no explanation in the NRC for how the energy value was derived. As fat became a more important energy source in ruminant diets during the 1980s and 1990s, energy values for fats came under greater scrutiny. Much of this was precipitated by development and marketing of "granular" fat sources that were designed to be convenient to handle and less likely to interfere with rumen fermentation. Companies marketing fat supplements typically included energy values in their product literature. Values varied, as did the origin of the values. Some were unexplained, some were derived primarily on a theoretical basis, and others were partially or totally based on research results. The focus of this paper is to describe how energy values for fat supplements fed to ruminants can be derived. After reading the discussion below, one should have an appreciation for the difficulty in deriving energy values and the amount of confidence one can have in the estimates.

## THE ENERGY SYSTEM

The net energy system used for describing the energy content of ruminant feeds is illustrated in Figure 1. To determine the gross energy (GE) content, the feed is totally combusted (burned) in an oven at 550°F. Mineral remains while the organic matter is converted to heat (energy) which can be measured.

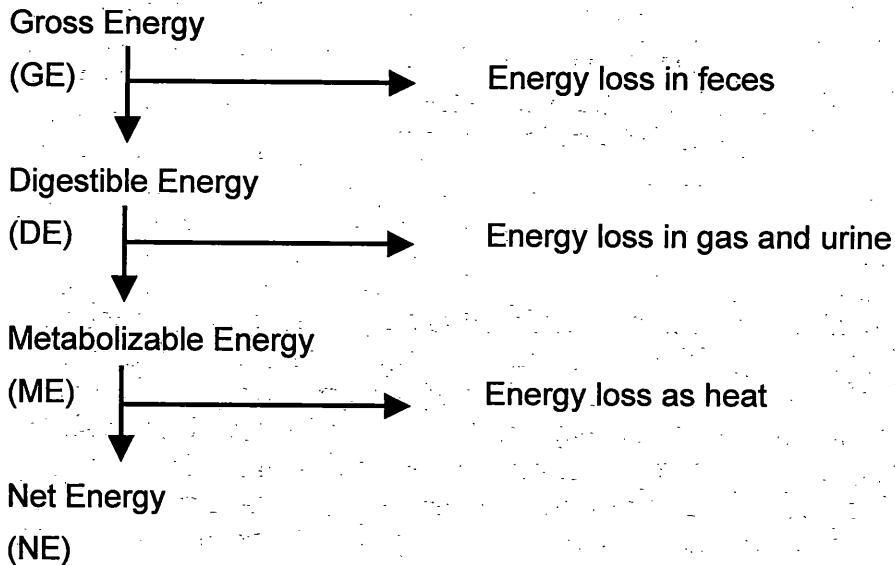


Figure 1. Net energy scheme

The GE content of feed is very easy and inexpensive to measure, but it is similar among feeds commonly used in ruminant diets and, therefore, not a very meaningful measurement. It is far more important to know what amount of the GE can ultimately be used by the animal for a productive function. That measurement is referred to as the net energy (NE) of the feed. Net energy is the energy that is available to the animal to maintain itself and to use for productive functions such as growth and milk production. The efficiency in which GE is converted to NE varies depending on what function the energy is used for. Therefore, different terms are used for describing the energy content of a feed depending on whether it is used for maintenance (NEm), growth (NEg), or lactation (NEl). In contrast to GE, NE is very difficult and expensive to measure and only a few laboratories in the world have the capability to make the measurement. As illustrated in Figure 1, to measure the NE content of a feed directly, energy losses in feces, gas, urine, and heat must be quantified. Since these are difficult measurements to make, other indirect methods have been developed for estimating the energy content of feeds.

## METHODS FOR ESTIMATING THE ENERGY CONTENT OF FEED

### Calorimetry

As previously indicated, the direct measurement of energy content of a feed involves quantifying energy losses in feces, gas, urine and heat following consumption of the

feed. Animals are placed in elaborate respiration chambers for collection of feces, gas, and urine. Energy released as heat is either measured directly (direct calorimetry) or is predicted from other measurements (e.g. gas and urinary nitrogen loss; indirect calorimetry). Two major problems occur when using this technique. Because it is expensive and equipment is limited, it is usually conducted using a few animals so there is minimal replication. Unfortunately, there is considerable animal variation in these measurements, so insufficient replication results in having low confidence in the results. Second, supplemental fat can not comprise the entire diet. Because fat is potentially toxic to rumen microbes, it must be fed as a relative minor component of the diet, usually less than 5% of diet dry matter. Typically, fat is added to the diet at the expense of corn. Therefore, the energy density of a fat supplement must be computed from the difference in energy concentration between diets with and without supplemental fat, adjusting for the estimated energy value of the corn that is replaced by fat. For example, if  $NEI_{test}$  = the measured NEI content of the test diet,  $NEI_{control}$  = NEI content of the diet with out supplemental fat, fat replaced corn at 3.0 % of dry matter, and the NEI value of the corn is 2.0 Mcal/kg (preestablished number derived from the literature, e.g., the appropriate NRC), then  $NEI_{supplemental\ fat} = [(NEI_{test} - NEI_{control}) / 0.03] + 2.0$ .

To my knowledge, calorimetry has only been used once for determining the energy content of a fat supplement fed to ruminants. The fat source was calcium salts of palm oil fatty acids (CS) and they were fed to dairy cattle (Andrew et al., 1991). The values obtained were 7.53 and 5.52 Mcal NEI/kg CS when supplementing lactation diets with 16 or 20% CP, or an average of 6.62 Mcal NEI/kg CS. This number was unrealistically high considering that 15% of CS is mineral which contains no energy. This equates to an NEI value of 7.79 (6.62/.85) Mcal/kg fatty acid. The unreasonably high value could be traced back to the GE estimate for CS that was determined by combusting each diet and doing a difference calculation similar to that described above for NE. The GE value obtained in this manner (8.98 Mcal GE/kg CS) was much higher than what was determined by combusting the fat supplement by itself (8.0r Mcal GE/kg CS). It was also much higher than the theoretical value (7.71 Mcal GE/kg CS) calculated from known GE values of the individual fatty acids comprising CS. However, the high GE value (8.98) was the starting point from which all other energy values were calculated from and, therefore, was reflected in the final estimate of NE. The high GE estimate probably reflects difficulty in mixing and sampling diets that contain only a small percentage of the test ingredient. It is also important to note that the standard error for the final NEI estimate for CS (6.62 Mcal/kg CS) was 1.74. This standard error is high and reflects the large amount of variation encountered when making these types of measurements.

### Estimates Based on Fat Digestibility

Because of the diverse selection of fat supplements available for diet formulation, it would be useful to compare their energy values. Clearly, there is not sufficient calorimetry data to allow such comparisons. As previously mentioned, GE values of fats can be obtained by combustion and measurement of heat or they can be calculated

based on their composition and known GE values of their components parts. Fatty acids are the energy rich component of fat supplements. The presence of non-fatty acid components in fat supplements, e.g. calcium in CS or glycerol when fatty acids are fed as a triglyceride, "dilutes" the energy value. Besides the GE value, the other major factor influencing the NE value for fat is the digestibility of the fatty acids. When fatty acids are fed, there is essentially no energy loss as gas because there is negligible fermentation of fatty acids in the gastrointestinal tract. Likewise, there are no energy losses in urine from feeding fatty acids. Consequently, the DE content of fatty acids equals the metabolizable energy (ME) content of fatty acids. There is energy loss as heat, but it is probably similar among fat sources. For example, in dairy cows, the efficiency of conversion of ME to NEI is assumed to be 80% for all fat sources (NRC, 2001). Consequently, once the GE content of a fat source is derived, determination of fatty acid digestibility becomes a powerful predictor of the NE content. The fatty acids that comprise fat supplements vary in chain length, degree of saturation, and structure (calcium salt vs. triglyceride vs. free fatty acid), all of which influence digestibility and, therefore, NEI (Grummer and Rebelo, 1998).

Obtaining digestibility coefficients for fat sources is not a trivial procedure. As mentioned above, the fat supplement of interest can not be fed as the sole ingredient in the diet. Therefore, similar to calorimetry experiments, fatty acid digestibility of a basal diet with and without fat is measured. Analytical separation of basal diet fatty acids from supplemental fatty acids is not possible, therefore, estimation of digestion coefficients for fat supplements is usually calculated based on the difference in fatty acid digestibility between the two diets and the amount of fatty acid added to the diet. A major assumption is that the fatty acid digestibility of the basal diet does not change when the test fat is added to the diet and, therefore, changes in fatty acid digestibility are accounted for by the test fat. Another assumption is that endogenous lipid flow and digestibility remains constant. These assumptions, particularly the former, may not be totally correct.

It is important to realize that digestibility (and hence NEI) of a fat source is not a constant value. Fatty acid digestibility is influenced by characteristics of the animal, basal diet, and level of feed intake (Grummer, 1992). Although not documented in ruminants, it is well documented in nonruminants that fat composition of the basal diet influences digestibility of supplemental fats (Grummer, 1992). Because fatty acid digestibility is not constant, it is wrong to compare the NEI value for fat "X" obtained from trial "A" to the NEI value for fat "Y" obtained from trial "B". Unless numerous studies have been conducted from which meaningful averages can be calculated, comparison of digestion coefficients and estimated NEI values among fat sources should only be done within a study to avoid "comparing apples to oranges".

Table 1 reports estimates of fat digestibility for several common sources used in dairy diets. Means were calculated from experimental observations from peer-reviewed scientific literature. Please note the large standard deviations for the mean of each fat source. This can be attributed to variability among experiments including composition of the basal diet, level of feed intake, physiological state of the animal, and experimental

methodology. While it seems plausible that there is a difference in digestibility between CS and partially hydrogenated tallow, the large standard deviations makes one less confident in making a similar statement regarding CS and hydrolyzed tallow fatty acids.

Table 1. Estimates of digestibility (by difference) for various fat sources when lactating cows are eating at approximately three times level of maintenance intake.

Parameter	Calcium salts of palm oil (n=15) <sup>1</sup>	Hydrolyzed tallow fatty acids (n=9)	Tallow. (n=10)	Partially hydrog. tallow (n=9)
Fatty acid digest. of supplement, %				
Mean	79.5	72.8	63.2	40.0
Std. Dev.	11.4	8.4	12.8	13.3
Range	55.9-92.7	59.1-83.7	44.3-80.7	14.2-56.8

<sup>1</sup>Number of experimental observations.

Digestibility of fat sources also has been estimated using a modeling approach (e.g. Moate et al., 2002). Several studies have been conducted using duodenally cannulated cows to determine digestion coefficients in the intestine for fatty acids varying in chain length and degree of saturation. For this proceeding, we summarized nine trials that were conducted (8 with lactating cows, 1 with steers) in which total and individual fatty acid intake and intestinal digestibility were measured. Data in Table 2 represent 40 treatment means and include control as well as fat supplemented treatments. The data indicate that the presence of one double bond accounts for an improvement in digestibility of unsaturated fatty acids vs. saturated fatty acids, i.e., intestinal digestibility of C18:1, C18:2 and C18:3 were very similar.

Table 2. Fatty acid intake and intestinal digestibility (nine trial summary).

	Total FA	C16:0	C18:0	C18:1	C18:2	C18:3
Intake, g/d						
n	40	40	40	35	40	27
Mean	833	171	58	250	240	53
Std. dev.	329	119	72	165	134	28
Intest. dig. (%)						
n	40	40	40	35	40	27
Mean	73	75	69	80	78	78
Std. dev.	11	8	15	9	11	13

By estimating the extent of hydrogenation of supplemental fatty acids in the rumen, one can predict the fatty acid profile reaching the duodenum. An overall digestion coefficient can then be calculated based on the proportion of each fatty acid reaching the duodenum and their corresponding digestion coefficient. Using this approach, digestion coefficients of supplemental fats are usually estimated to be much higher than those obtained by difference. Using the data in Table 2, no fat source would be predicted to have a digestion coefficient below 69% since all fatty acids appear to have digestion coefficients equal to or greater than that. However, digestion coefficients determined by difference for hydrogenated tallow are typically reported to be far below 69% (Table 1). Elliott et al. (1999) reported that triglycerides that are very saturated are not readily

hydrolyzed in the rumen. It is possible that saturated fatty acids reaching the intestine in esterified form are less digestible than those delivered as free fatty acids. Results from trials examining differences in digestibility of saturated free fatty acids vs. saturated esterified fatty acids is mixed (Elliot et al., 1999, Grummer and Rabelo, 1998). If there are differences they appear to be subtler than those needed to explain the low digestibility of hydrogenated fat supplements derived by the difference method. Once estimates of fatty acid digestibility are obtained, estimates of NE can be calculated in a similar fashion to that used by the NRC (2001). First, the digestible energy (DE) value of the supplement at maintenance is calculated:

For fat sources that contain glycerol (fatty acids present as triglyceride):

$$DE_{1x} (\%) = (9.4 \times FAdig \times 0.9 \times (EE/100)) + (4.3 \times .1 \times (EE/100))$$

The triglyceride in the ether extract (EE) of these supplements contain 10% glycerol and 90% fatty acid and it is assumed that glycerol is 100% digestible. Gross energy content of fat and carbohydrate is 9.4 and 4.3 Mcal/kg, respectively. Fatty acid digestibility is for an animal at a maintenance level of feed intake (an 8% increase in digestibility compared to when an animal is at three times level of maintenance intake, e.g., those values in Table 1).

For fat sources without glycerol:

$$DE_{1x} (\%) = 9.4 \times FAdig \times (EE/100)$$

This equation also works for CS because EE would = 85 and the remaining portion of the supplement is calcium, which does not contain energy.

The DE content at production level of intake (DE<sub>p</sub>) can then be calculated as described on page 14 of the NRC (2001) for dairy cattle.

Then ME<sub>p</sub> = DE<sub>p</sub> and NE<sub>l</sub>p = .8 x ME<sub>p</sub> (Mcal/kg)

Fatty acid true digestibility values adjusted to maintenance level of feed intake and NE<sub>l</sub> values assuming intake at three times maintenance for several fat sources are shown in Table 3. Literature values for digestibility of vegetable oil were not available, therefore, digestibility was assumed to be 80% at maintenance and 86% at three times maintenance level of intake.

Table 3. Digestibility values for fat supplements at maintenance level of intake and corresponding NE<sub>l</sub> values at three times maintenance (NRC,2001).

Fat Source	Mean Digestibility Coefficient	NE <sub>l3x</sub> (Mcal/kg)
CS	.86	5.02
Tallow	.68	4.53
Hydrolyzed Tallow Fatty Acids	.79	5.41
Partially Hydrogenated Tallow Fatty acids	.43	2.97
Vegetable Oil	.86	5.65

## Estimates Based on Animal Performance

For beef cattle, NEm and NEg values for supplemental fats have usually been derived from feeding trials in which animal performance was monitored while feeding diets with and without supplemental fat (Zinn and Plascencia, 1996). Energy for maintenance (= average daily gain<sup>1.095</sup> x (.0557 x body weight<sup>.75</sup>)) and energy for gain (= .077 x body weight<sup>.75</sup>) are estimated from equations previously derived from the literature. From these estimates, NEm and NEg values for diets with and without fat were obtained by process of iteration (Zinn and Plascencia, 1996) to fit the relationship: NEg = .877NEm - .410, NRC (1984). Once the NEm and NEg values of the control and fat supplemented diet are estimated, the corresponding NEm and NEg values for fat can be obtained by doing difference calculations as described above for calorimetry experiments.

Energy values for several fats fed to growing beef cattle are shown in Table 4. Values vary considerably and may reflect differences in basal diet fed, fatty acid composition of test fat, physiological state of the animal, level of feed intake, or level of fat intake. Zinn (1994) reported that the NEm value of fat supplements decline 1.75 Mcal/kg per gram of increase in lipid intake/kg BW.

Table 4. Estimates of the NEm and NEg value of fats fed to growing beef cattle.

Fat Source	NEm	NEg
Yellow Grease (Zinn, 1988)	6.20	4.53
Yellow Grease (Zinn, 1989)	6.03	4.79
Animal-Vegetable Blend (Zinn, 1989)	5.53	4.43
Soybean oil (Brandt and Anderson, 1990)	4.48	3.56
Tallow (Brandt and Anderson, 1990)	5.05	4.13
Yellow Grease (Brandt and Anderson, 1990)	5.34	4.13
Tallow (Clary, 1993)	2.95	---
Yellow Grease (Zinn and Plascencia, 1996) [10% forage diet]	5.71	4.65
Yellow Grease (Zinn and Plascencia, 1996) [30% forage diet]	3.55	2.65
Yellow Grease (Zinn and Shen, 1996)	4.78	3.87
Yellow Grease/Griddle Grease (Plascencia et al., 1999)	4.98	3.95

## Conclusions

Several critical points:

1. All fat sources do not have the same energy value.
2. It is not realistic to assign a single NE value to a fat source. Values may vary depending on basal diet, level of intake, and physiological state of the animal.
3. Determining NE values for fat supplements is difficult and values available in the literature are extremely variable.
4. Comparative NE values for commonly fed fat sources would be useful for diet formulation, however, they are difficult to obtain through the literature. There are a paucity of trials that make head to head comparisons among fat sources. Pooling

values across the literature to derive mean values for each fat supplement yields high standard deviations.

5. Modeling lipid digestion and absorption may be a plausible approach to obtain comparative energy values for fat supplements, but models must be dynamic enough to compare fats under a wide variety of conditions.

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# **THE USE OF BLOOD COMPONENT ANALYSIS IN NUTRITURE ASSESSMENT OF DAIRY CATTLE**

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## AIM OF PRESENTATION

The aim of this presentation is to discuss in general the use of blood analysis for nutriture assessment. The utility, challenges, sampling strategies, and methods of interpretation will be discussed collectively with only a brief mention of specific analytes as examples. A table including some information about a few specific analytes is included at the end of this paper.

## CONCEPT OF "NUTRITURE ASSESSMENT"

*Nutriture* is defined as "the status of the body in relation to nutrition, generally or in regard to a specific nutrient..." (Dorland's Illustrated Medical Dictionary, 24<sup>th</sup> ed.) I don't think this is a foreign concept to anyone in animal production. Important variables in nutriture assessment include growth rate, body condition, back-fat thickness, milk production, and many others. These are all measures of nutriture assessment. Measurement of blood component (analyte) concentrations is an additional means of nutriture assessment. Again, I don't think this is a foreign concept. Literally thousands of research papers could be cited in which nutritional treatments have been evaluated, at least in part, by responses in blood composition. The challenge is in application of blood analysis for nutriture assessment under commercial production situations. The major considerations are in the selection of analytes to measure, and in the interpretation of results. There are both biological and statistical considerations in the interpretation of blood analyte concentrations for nutriture assessment.

## BIOLOGICAL FACTORS IN THE NUTRITIONAL INTERPRETATION OF BLOOD COMPOSITION

An understanding of the physiology and metabolism of the blood analytes measured is essential for appropriate nutritional interpretation. Interpretation is often not straightforward. One critical biological factor with a large influence on interpretation is the presence or absence of disease.

## THE DIFFERENCE BETWEEN DISEASE DIAGNOSIS AND NUTRITURE ASSESSMENT

There is an important distinction between the use of blood analysis for disease diagnosis and for nutriture assessment. First, disease diagnosis is intended to measure pathological (abnormal) variation, which is usually much greater than physiological (normal) variation. It is generally physiological variation that is of interest in nutriture assessment. Much more important, however, is the effect that disease, be it infectious, metabolic, toxic or other, can have on a wide variety of blood component concentrations.

Figure 1 represents the results of a blood chemistry analysis done on a down fresh cow. The scale is arbitrary, but the light gray boxes represent the “normal range,” as defined by the clinical pathology laboratory. The darker lines represent the values measured in this animal. Note in this example, all of the values are outside of the defined normal ranges. In looking at these results, we might suppose there are important nutritional problems with this animal, and with the herd from which she came. The NEFA concentration is too high, indicating negative energy balance. The zinc concentration is too low, suggesting a dietary zinc deficiency. We could go on to suppose from these results that there are numerous nutritional problems. In fact, it is quite likely the cow has milk fever and her primary problem is hypocalcemia. All of the other abnormalities in this figure could be explained as secondary effects to hypocalcemia. If, based on these results we added zinc oxide to the ration of this herd, we probably made a mistake!

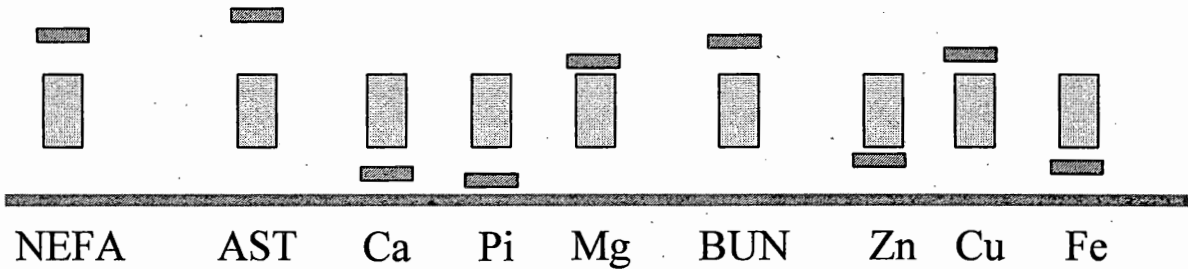


Figure 1. Blood chemistry analysis from a single cow, down after freshening.

Contrast the previous situation with Figure 2. In this case, light gray boxes still represent a reference or “normal range,” but the dark lines represent the average values from seven apparently healthy cows. Note that all of the values are in the reference range, except for urea nitrogen (BUN). Elevated BUN can be a sign of severe dehydration or kidney disease, however it is quite unlikely that seven apparently healthy cows are all severely dehydrated or have kidney disease. It is much more likely that the high BUN represents a physiological variation and the values are in this range due to absorption of large amounts of ammonia from the rumen. It is quite possible that this herd would benefit from an adjustment in the ratio of rumen available-energy to rumen available-nitrogen.

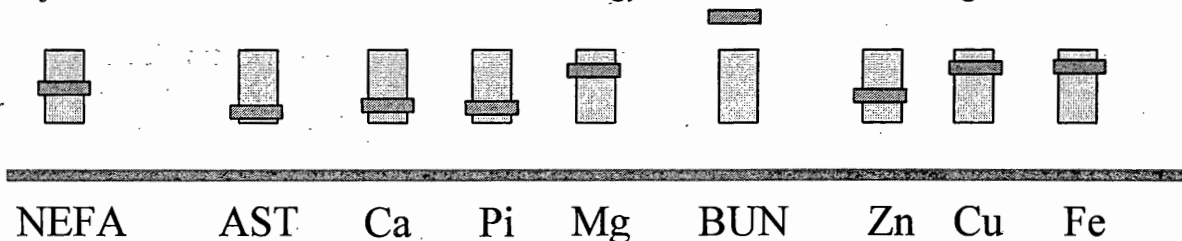


Figure 2. Average values of serum chemistry analyses from seven apparently healthy cows in a single herd.

The major point of this example is that samples taken from diseased animals cannot be used to make general nutritional inferences. Even in those cases in which animals have what appears to be a nutritional disease, samples should be taken from other apparently healthy animals to verify that the problem extends beyond a single individual, and is therefore likely to be related to diet.

## FACTORS AFFECTING PHYSIOLOGICAL VARIATION ARE OFTEN COMPLEX

In some cases, such as that for selenium, the concentration in the blood or blood serum is a reasonably direct indicator of dietary intake. For many other metabolites and minerals, the relationship between nutrition and blood concentration is more complex. The serum concentration of iron is a good example. Dietary iron intake can affect serum iron concentration, but so can other factors. One is the protein status of the animal. Serum iron is carried on a short-half life protein known as *transferrin*. The serum iron concentration is affected by the availability of transferrin, which is in turn related to protein status of the animal, among other things. Therefore, a low serum iron concentration in an apparently healthy animal might mean low iron status, but it might also mean a low protein status. Many similar examples exist, making it important to understand the basic metabolism of each blood analyte measured.

## STATISTICAL CONSIDERATIONS IN THE NUTRITIONAL INTERPRETATION OF BLOOD COMPOSITION

Results of controlled research projects prove that diet and nutriture affect blood composition. However, *controlled research* implies that careful measures have been taken to minimize extraneous variation, thus maximizing the likelihood that a treatment effect will be detected, if one exists. Farm conditions don't resemble the conditions under which controlled research is conducted and the challenge in using blood component analysis for nutriture assessment under commercial conditions is in minimizing extraneous variation.

To understand this challenge, let's first consider the sources, or components, of variation in blood composition of animals. These could be listed as follows

- Random biological variation
- Genetic variation
- Circadian and/or prandial variation
- Seasonal variation
- Variation associated with physiological state (growth, gestation stage, lactation stage, etc.)
- Variation associated with pathological state (the effect of existing disease)
- Artifactual variation due to sampling or sample handling technique
- Analytical variation
- Environmental variation (influences external to the animal, including nutrition and other management factors)

Of this list, it is only the last item in which we are interested for nutriture assessment. All of the other sources of variation are extraneous to the purpose of nutriture assessment.

One of the ways in which extraneous variation can be limited is through sampling strategy. This involves primarily the selection of animals and the timing of sampling, but should also include the type of sample taken and the way in which it is handled.

Pathological variation, as mentioned above, is controlled by selecting only apparently healthy animals, and also by sampling several animals because it is unlikely that all will

be suffering from a disease. Grouping animals by physiological state, such as age or lactation stage, controls physiological variation. Collecting blood samples at a fixed time relative to feeding controls for prandial variation, and careful attention to proper sample handling should eliminate variation due to sample handling errors.

Variation due to genetics, analytical technique, and unexplained biological randomness is not subject to control under farm conditions. However, sampling multiple animals can minimize the effects of these sources of variation on the interpretation of test results. The optimum number of animals to test is seldom known precisely, and indeed will vary with the user's definition of *optimum*. However, testing seven animals is a reasonable thumb rule, when more specific information about a given analyte is not available. Remember, this number is to be applied after the grouping has been decided upon. Three animals from one age group and four from another doesn't constitute a group of seven animals in this context; seven should be sampled from each group! If analytical cost is a major concern, the most critical group should be selected for sampling, rather than skimping on the number of animals per group.

#### VARIABILITY CHARACTERISTICS ASSOCIATED WITH SPECIFIC ANALYTES

There are variability characteristics associated with each specific blood analyte that give clues to its potential usefulness in nutriture assessment. One of these characteristics is the physiological range. The blood concentration of some analytes is under strict homeostatic regulation. In these cases, there is a relatively narrow physiological range and homeostasis limits the effect that environment (including nutrition) can have on the blood concentration. In figure 3 note that there appears to be much less rigid homeostatic control of blood NEFA, compared to blood glucose. This suggests that blood NEFA has greater potential for nutriture assessment than glucose. The breadth of the physiological range is related to the coefficient of variation for the analyte. Coefficients of variation for selected analytes are given in the tables at the end of this paper.

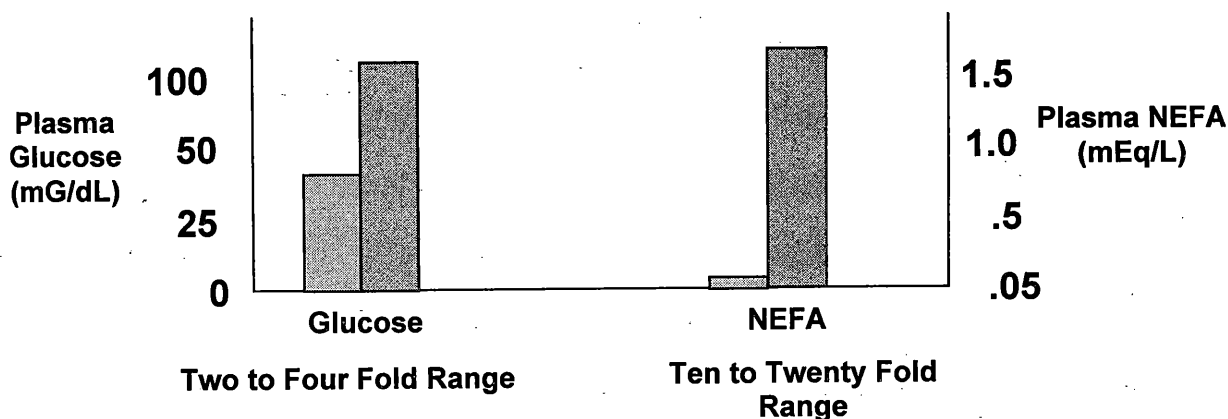


Figure 3 Comparison of the physiological range of glucose and NEFA (light bar = lower limit, dark bar = upper limit). Notice the much larger normal range for NEFAs compared to glucose. Thus there is relatively less homeostatic regulation of plasma NEFA concentration, compared to glucose. This allows environment (including nutrition) to play a larger role in blood NEFA concentration, compared to blood glucose.

Examination of variability components gives another hint to the potential usefulness of various analytes for nutriture assessment. Statistical techniques exist that divide variance into components. The concept of variability components is illustrated in Figure 4. With respect to nutriture assessment, an important variance component is the proportion of variability due to herd. When this proportion is high it means 1) there is a large effect of environment (probably nutritional) on the blood value in question and 2) there is a reasonable chance of detecting change with a relatively small number of samples. A list of variance components due to herd for some select analytes is given in the tables at the end of this paper

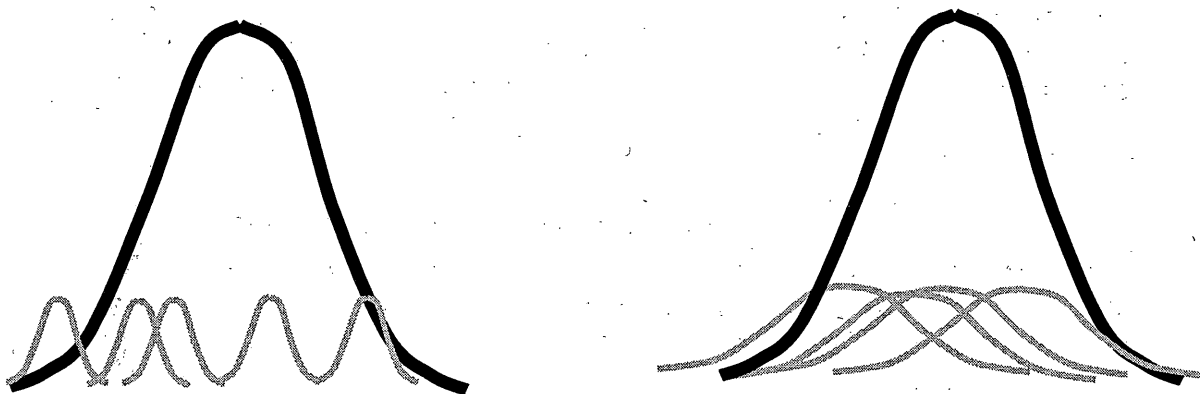


Figure 4. An illustration of variance components. The large curves represent the population distribution for a variable, and the small curves the distribution within individual herds. In the figure on the right, there is a great deal of variability within herds, resulting in substantial overlap of the individual herd distributions. Variability within herd accounts for a major portion of the population variability, making variability due to herd a small portion of the total. In the figure on the left there is a similar population distribution, but here there is relatively little variation within herd and a large proportion of variability due to herd. Blood analyte concentrations with distribution characteristics like the figure on the left have much greater potential for nutriture assessment than do those distribution patterns like the figure on the right.

### INTERPRETATION

Interpretation of blood analyte concentrations for nutriture assessment requires that there be some frame of reference available, i.e. a scale by which to evaluate the results of a test. Numerous textbooks exist with reference ranges for clinical chemistry variables for cattle. These include such standard reference texts as the Merck Veterinary Manual. The reference ranges given by such texts are intended to be used in disease diagnosis of individual animals and they are based on the need to exclude a very high portion of normal individuals. They are of little usefulness in nutritional assessment.

An alternative to the use of frequency distributions from individual animal values for the construction of reference ranges would be to use frequency distributions of herd means. A comprehensive listing of such ranges, to my knowledge does not exist, and if it did there would be constant concern about the appropriateness of the population of herds from which they were based. For example, if the herd in question has a 30,000-pound

rolling-herd average, is it appropriate to compare it to a reference range based on herds of lower milk production.

Perhaps a better means of establishing reference ranges is by considering correlative information comparing blood analyte concentrations to nutritionally related outcomes. One outcome of particular interest in dairy cows is the incidence of peripartum metabolic diseases. In this kind of a comparison, blood analyte concentrations are analyzed as “risk factors” for a disease. Risk factors can be negative or positive, indicating that higher blood concentrations of the analyte in question either decrease or increase the risk of disease, respectively.

Table one below is an example of a multiple logistic regression. These are the final variables in a model that was built from seven original variables. The data were from 1170 cows distributed among 67 dairy herds in Michigan (Cameron et al., 1998). Cows were classified as having elevated plasma NEFA concentrations if values were above 0.3 mEq/L. The analysis says that dry cows having plasma NEFA > 0.3 mEq/L are slightly more than twice as likely to develop a displaced abomasum after calving than dry cows with plasma NEFA less than 0.3 mEq/L. The exact value of 0.3 mEq/L is not as important as the observation that lower NEFA is good and higher NEFA is bad, with respect to the occurrence of displaced abomasum.

Table 1. Multivariable logistic model with random effects for individual cows for the occurrence or nonoccurrence of displaced abomasum.

Variable	Sign	P	Risk Ratio
Body Condition Score	pos	0.001	2.405
Winter season	pos	0.002	2.967
Elevated NEFA	pos	0.007	2.042
Lactation number	neg	0.045	0.8075

This general approach needs to be applied to additional blood analytes and additional diseases, but is far more likely to yield meaningful interpretations than are other statistical methods.

Another approach to the evaluation of blood analysis for nutriture assessment is the use of *Statistical Process Control* (SPC) charts. Use of SPC has been applied recently to the evaluation of blood variables in dairy herds. This is a fairly simple and straightforward technique that may have application in larger herds. The utility of SPC is in its ability to track changes within a herd and to separate variance components. Figure 5 is an example of a *process control chart*, the basic tool of SPC. This example chart is of cholesterol concentrations in pre-fresh cows in a large Michigan dairy. There is not time in this presentation to discuss SPC in detail. Briefly, each point on the chart is the mean of seven animals sampled on the same day. In this case samples were taken weekly, and different animals sampled each week. The central line is the grand mean of all samples. The two dotted lines are referred to as *control limits*. When values extend past the control limits, it indicates the presence of an additional variance component, i.e. the

change is significant. Variation within the control limits is considered to be random and to occur due to variance components that are constant within the herd.

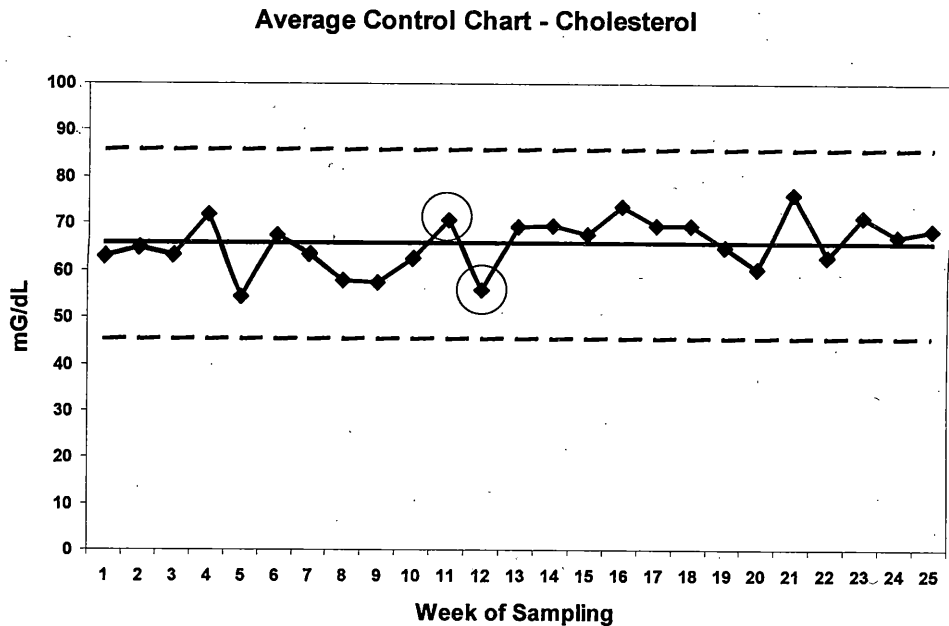


Figure 5. Process control chart based on average serum cholesterol concentrations from pre-fresh cows in a large Michigan dairy. Each point represents the mean of seven animals sampled at the same time. The solid line is the grand mean of all the samples. The two dotted lines are the upper and lower control limits. The upper and lower control limits are functions of the inherent variability in the system, and the number of animals tested per point. Weekly points falling outside of the control limits indicate that an additional variance component has been added, i.e. that something has changed and caused the variation. Variability among points within the control limits is considered random variation inherent to the system, i.e. one should not look for “causes” of variation within the control limits.

An important point to keep in mind about process control charts is that the control limits do not represent anything biological, i.e. it is not inherently good or bad that values are outside the limits. It only means that something has changed. Whether the change is favorable or unfavorable depends on a biological interpretation that requires some idea of what range of values is desirable. Process control charts appear to offer a potentially powerful way to monitor nutrition through blood sampling. They appear to be most useful in large herds where there are adequate numbers of animals for optimal groupings, there are sufficient animals over which to spread the testing cost, and the cost-benefit ratio is potentially very high.

## SPECIFIC ANALYTES

### Metabolites and Organic Components

At the Diagnostic Center for Population and Animal Health (formerly the Animal Health Diagnostic Laboratory) at Michigan State University we offer a *metabolic profile* to assess certain aspects of the metabolic and nutritional status of *transition cows*, defined as cows between three weeks before and three weeks after calving. Analytes in this profile consist of plasma non-esterified fatty acids (NEFA), beta-hydroxybutyric acid (BHB, a ketone body), albumin, urea nitrogen (SUN, or BUN), and aspartate aminotransferase (AST). These analytes were chosen because of their specific utility in the evaluation of cows in this period. A summary of their characteristics for nutriture assessment is in Table 2. Other organic analytes in blood may be useful in other situations.

### Minerals.

There is generally a large interest in assessment of mineral nutriture based on blood samples. Important considerations in the utility of blood mineral concentration in nutriture assessment are the relative degree of homeostatic control of serum concentrations, and the means by which homeostatic control is achieved. For some minerals homeostasis is regulated at the level of absorption. Examples are calcium, iron, and zinc. Absorption of these minerals is adjusted to meet body needs. When nutritional status is adequate, absorption of the mineral from the gut is nearly shut down. Thus, the mineral never makes it into the blood to affect serum concentrations. In the case of minerals such as these, blood concentrations are generally poor indicators of nutritional status.

This is in contrast to minerals like selenium and magnesium. These minerals absorbed in a relatively unregulated manner and homeostasis is achieved by renal excretion of the excess mineral. In these cases, blood or serum concentrations are good indicators of nutriture because the excess mineral must travel through the blood on its way to renal excretion.

The Toxicology Section of the Diagnostic Center for Population and Animal health offers a battery of mineral analyses as a panel. The potential utility for nutriture assessment for serum concentrations of some minerals in the panel is listed in Table 3.

## SUMMARY

Analysis of blood samples has variable utility for assessment of nutriture, depending on the specific analyte in question. The major challenges in applying blood sampling as a nutriture assessment technique in the field are in minimizing extraneous variation. Important techniques for minimizing extraneous variation are selecting apparently healthy animals, grouping animals for sampling, sampling at fixed times with respect to feeding and milking, and sampling multiple animals (usually at least seven per group). Rigid reference ranges are not known, and may not be knowable. Associative statistical techniques, such as logistic regression, may be very useful in evaluating specific analytes for their utility in nutriture assessment, and in establishing reference values. Additional,

on-farm statistical techniques are needed to aid in the interpretation of blood results. When picking out blood analytes for testing, it is important to consider metabolic and physiological factors influencing serum concentrations, the variability characteristics, the type of sample needed, and the sample handling necessary to achieve accurate results.

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Herdt TH, Rumbleha W, Braselton WE. 2000. The use of blood analyses to evaluate mineral status in livestock. *Vet. Clinics of North America, Food Animal Practice.* 16: 423-444.

Table 2. Analytes in the Michigan State Transition Cow Profile. Contact the laboratory at 517 353 9312 for information on submitting samples.

Analyte	Nutritional Implication	Grouping Strategy	Sampling Considerations	Reference values	Coefficient of variation	Percent of variation due to herd
Beta hydroxy-butyric acid (BHB, a ketone body)	Concentrations are normally elevated in early lactation, compared to other times. Concentrations increase excessively in the presence of large negative energy balance in combination with low availability of glucose precursors.	Select seven healthy appearing animals in the first 3 to 4 weeks of lactation	Serum is the blood sample of choice. Chill samples. Concentrations are stable in chilled or frozen serum. Avoid hemolysis. In cattle without subclinical ketosis, values are highest 2 to 4 hours post feeding.	Concentrations greater than 10 to 12 mg/dl are consistent with subclinical ketosis. In well nourished herds it should be possible to maintain a subclinical ketosis prevalence of less than 20%	Early lactation: 33% Mid lactation: 34% Dry cows: 40%	Early lactation: 25% Mid lactation: 40% Dry cows: 43%
Albumin	In healthy animals, serum concentration is related to labile protein stores.	Select seven healthy appearing animals in the first three to four weeks of lactation, or the last week of gestation.	Serum is the blood sample of choice. Chill samples. Concentrations are stable in chilled or frozen serum. Avoid hemolysis.	Concentrations normally decline near parturition, so reference values based on cattle at other times are too high. Values in the range of 3 g/dL or above are consistent with good protein stores; values less than 2.5 g/dL are consistent with inadequate protein stores		

Table 2. Continued.

Analyte	Nutritional Implication	Grouping Strategy	Sampling Considerations	Reference values	Coefficient of variation	Percent of variation due to herd
Aspartate aminotransferase	Sensitive, but not specific, indicator of fatty liver infiltration	Select seven healthy appearing cows in the last three weeks of gestation, or first three weeks of lactation	Serum is the sample of choice. Activity is relatively stable in serum. Chill quickly after collection and freeze before sending to laboratory	Desirable values are less than 100 IU/L. Values are somewhat laboratory specific		
Urea Nitrogen (BUN)	Serum concentration is correlated with rumen ammonia concentration. Values are indicative of the balance of rumen available carbohydrate and nitrogen.	Seven animals in the same feeding group.	Serum is the blood sample of choice in non-lactating animals. Chill samples. Concentrations are stable in chilled or frozen serum.	Between 13 and 16 mG/dL		

Table 2. Continued.

Analyte	Nutritional Implication	Grouping Strategy	Sampling Considerations	Reference values	Coefficient of variation	Percent of variation due to herd
Non-esterified fatty acids (NEFA)	Indicator of negative energy balance	Seven cows in the last 3 weeks of gestation, or first 3 weeks of lactation. Collect samples just before the normal time at which fresh feed is offered.	Samples should be chilled immediately upon collection. Plasma is a convenient sample because cells can be separated easily from chilled samples. Values are stable in frozen plasma.	For dry cows between three weeks and three days before calving, goal concentrations are less than 0.3 mEq/L. For fresh cows more than 3 days in milk, goal values are less than 0.7 mEq/L.	Late gestation: 87% Early lactation: 110%	Late gestation: 43% Early lactation: 54%

Table 3. Selected minerals available in the Michigan State *Serum soluble elements* profile. Contact the laboratory at 517 355 0281. Coefficients of variation and herd variance components are from Herdt et al, 2000.

Analyte	Nutritional Implication	Grouping Strategy	Sampling Considerations	Reference values	Coefficient of variation	Percent of variation due to herd
Blood Selenium	Good indicator of nutritional adequacy	Seven animals of similar age	Uncoagulated blood, EDTA is a sufficient anticoagulant	For individual adults: 120 to 250 nG/mL Target herd mean: 185 nG/mL (ppb)	14.5%	69%
Copper	Very low serum concentrations are indicative of nutritional inadequacy, however serum copper is not a sensitive index of copper nutriture	Thirteen apparently healthy animals of similar age (Herdt et al., 2000)	Serum is the sample of choice	For individual animals: 0.6 to 1.0 mG/L (ppm) Target herd mean: 0.8 mG/L (ppm)	17%	33%
Zinc	Very low serum concentrations are indicative of nutritional inadequacy, but serum zinc is a very insensitive indicator of zinc nutriture	Ten apparently healthy animals of similar age	Serum is the sample of choice. Avoid contact with the usual types of rubber tube stopper. Use BD "royal-blue" stopper tubes	For individual animals: 0.8 to 1.4 mG/L (ppm) Target herd mean: 1.1 mG/L (ppm)		

# IMPLANT STRATEGIES FOR DAIRY STEERS

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## INTRODUCTION

The availability of growth promotant options on the market today (Table 1) targeted to cattle ranging from young calves to growing and finishing steers provides dairy steer producers the flexibility of designing an implant strategy to meet their goals for an economic return for a specific market niche. Expectations in typical beef feed yards are for a return of \$10 to \$30 per dollar invested in growth promotants (Anderson, 2001). In an evaluation of 37 steer trials, Duckett et al. (1996) observed that implanted cattle increased daily gains 18%, feed intake 6%, feed efficiency 8%, carcass weight 5%, and rib-eye area 4% compared to those receiving no implants. Overall carcass traits and tenderness were not significantly affected negatively by implanting although implanted cattle had a 14.5% decrease in the percentage choice. The net return was \$18.32 in cattle sold live and \$13.53 on a grade and yield basis per implant investment, the latter being offset by decreases in choice grades. Guiroy et al. (2002) found that although anabolic implants improved the efficiency of absorbed energy, the finished body weight of steers to reach the same body composition as non-implanted increased from 30 to 90 lbs, depending on the implant strategy used. If producers decide that they will not use implants because of a specific market niche and consumer preference, then a premium of \$30 to \$60/steer will be necessary to make this option economical (Anderson, 1998).

The challenge with long-fed dairy steers is to integrate a suitable implant strategy with feeding programs at each phase of production to maintain an economic return. This paper will review general recommendations for implanting strategies for feedlot cattle under varying nutritional regimens with application to dairy steers in the feedlot and on-pasture. The focus will be on Holstein steers.

## MECHANISM OF ACTION

To attain the full benefit of growth promotants all implants must be administered in the middle third of the back side of the ear or in the last third of the ear if part of the ear is lost through frost bite (Griffin and Mader, 1997). Incorrect placement of the implant causing implants to be lost or abscessed implant sites will reduce daily gain by an average of 0.13 lbs (Berry et al., 2000). A quality assurance implant placement program is an important consideration to improve the consistency of the response to implants. An example known as zero defect implanting which has reduced implant defects and abscesses was discussed by Cook (2000). Cattle must be fed to meet their nutrient requirements (NRC, 1996) for projected performance during the growing and finishing period for implants to be effective.

The basic mechanism of action of growth promotants relates to either their estrogenic or androgenic activities or a combination of both. Feed intake is typically increased by estrogenic implants resulting in enhanced gain in addition to changes in body composition. It has been suggested that the effects of estradiol are mediated through alteration of the somatotrophic axis with increasing levels of circulating somatotropin (ST) and insulin-like growth factor-1 (IGF-1). The release of growth promotant from an implant declines after a few days but is maintained at a high enough effective level to stimulate a growth response. Implants vary in the length of time they remain effective for growth stimulation which is the premise of re-implantation strategies (Griffin and Mader, 1997; Cook, 2000). The synthetic androgen, trenbolone, is approved for steers and heifers in the form of trenbolone acetate (TBA). Trenbolone acetate is a synthetic steroid with similar structures to both testosterone and estradiol. Trenbolone binds to both testosterone and estrogen receptors in muscle and other tissue (Anderson, 1991). Androgens have a direct effect on muscle cells which result in a net increase in protein accretion and have an indirect benefit of interfering with anti-anabolic effects of corticosteroids competing for corticosteroid binding sites (Cook, 2000). Trenbolone has no direct effect on adipose tissue but will reduce fat deposition by altering nutrient partitioning. The combination of TBA with estradiol (E) or zeranol (synthetic estrogen) enhances the growth, efficiency of feed nutrient utilization, and muscle deposition in steers (Anderson, 1991).

## IMPLANTS AND NUTRIENT INTERRELATIONSHIPS

### *General perspective:*

The effect of anabolic implants on nutrient requirements are accounted for by the relationship to protein, fat and energy accretion at a constant body composition and finished body weight. NRC (1996) indicated that protein content of gain equivalent to a 77 lb change in final shrunk body weight (FSBW) results from using estrogenic implants and 154 lb equivalent change in FSBW for the combination of TBA and E compared to not using an implant. Evaluation of the effect of 120 mg TBA in combination with 24 mg E (Revalor S®) vs. no implant administered to large frame 867 lb cross-bred steers by Johnson et al. (1996) showed a 82% increase in carcass protein accretion during the first 40 days after implanting. There were no effects on carcass fat deposition except a lower kidney, pelvic and heart (KPH) fat accumulation. Net energy requirements for gain have been reduced by at least 5% when anabolic implants are used (NRC, 1996).

Guiroy et al. (2002) in a summary of 13 studies with 13,640 cattle (66% steers), calculated the adjusted FSBW at a 28% empty body fat final BW (AFBW) and observed that the response to anabolic implants is due to a combination of decrease in the proportion of dry matter intake (DMI) needed for maintenance, reduced energy content of gain and efficiency of use of absorbed energy. Implant strategy did reduce the percentage of steers grading low choice compared to non-implanted steers. In addition to effect of implants, considerations for use of ionophores (additive response with implants), previous plane of nutrition, environmental conditions and frame size/breed effects are accounted for by NRC (1996) and refined by Cornell Cattle Systems 5 (CCS5; 2002).

Holstein steers are very sensitive to environmental stressors such as high temperatures and humidity, low temperatures with wet hair coat, wind speed with wet hair coats, and muddy conditions (Chester-Jones et al., 1998). These conditions affect DMI and performance and reduce effectiveness of an implant program. The maintenance energy (NE<sub>m</sub>) requirements of Holstein steers can be reduced by use of implants (Ainslie et al., 1992). They found that NE<sub>m</sub> for steers receiving a Revalor® implant was 60 kcal per unit of metabolic BW (MBW) compared to 77 kcal per unit of MBW for non-implanted steers. Holstein steers that have the propensity for compensatory gain (such as feeding a high energy diet following a period of higher roughage feeding) will improve energy utilization for both NE<sub>m</sub> and NE<sub>g</sub> which may be further enhanced by implant strategies (NRC, 1996).

Table 1. Selection of implant products available for growing and finishing steers<sup>a</sup>

Brand Name	Estrogen mg/implant	Progesterone (P) mg/implant	Androgen mg/implant	Re-implant Window, d	Estimated Pay-out, d
<i>Lower Potency</i>					
Ralgro®	36 mg zeranol <sup>b</sup>	-----	-----	45 to 90	70 to 100
Synovex C®	10 mg estradiol benzoate (EB) <sup>c</sup>	100 mg P	-----	45 to 90	100 to 140
Component E-C®	10 mg EB	100 mg P	-----	45 to 90	100 to 140
Calfoid®	10 mg EB	100 mg P	-----	45 to 90	100 to 140
<i>Moderate Potency</i>					
Finaplix S®	-----	-----	140 mg TBA <sup>d</sup>	70 to 100	60 to 100
Component T-S®	-----	-----	140 mg TBA	70 to 100	60 to 100
Revalor IS®	16 mg E	-----	80 mg TBA	120	100 to 140
Revalor G®	8 mg estradiol -17 β (E)	-----	40 mg TBA	120	100 to 140
Synovex S®	20 mg EB	200 mg P	-----	70 to 100	100 to 140
Component E-S®	20 mg EB	200 mg P	-----	70 to 100	100 to 140
Implus S®	20 mg EB	200 mg P	-----	70 to 100	100 to 140
Compudose®	25.7 mg E	-----	-----	140 to 170	170 to 200
Encore®	43.9 mg E	-----	-----	140 to 170	300+
<i>Higher Potency</i>					
Magnum®	72 mg zeranol	-----	-----	70 to 100	100 to 120
Revalor S®	24 mg E	-----	120 mg TBA	90 to 100	100 to 140
Revalor 200®	20 mg E	-----	200 mg TBA	90 to 100	100 to 140
Component TE-S®	24 mg E	-----	120 mg TBA	90 to 100	100 to 140
Synovex Plus®	28 mg EB	-----	200 mg TBA	90 to 100	100 to 140

<sup>a</sup>Adapted from Griffin and Mader (1997), Anderson (1998) and Loy (2001).

<sup>b</sup>Zeranol contains 30-33% the estrogenic activity of estradiol-17β

<sup>c</sup>Estradiol benzoate is 72% the estrogenic activity of estradiol-17β

<sup>d</sup>TBA = trenbolone acetate

Feedlot implant strategies are based on target market dates, genetic potential of the cattle, predicted changes in market prices and feed costs. If low choice grade is 28.6% empty body fat then the relationship between implanted cattle, frame size and BW to attain low choice marbling grade is shown in Table 2. Implanting changes the growth curve upward to a higher level. For example implanted cattle of frame score 5 will have to be fed out to a heavier BW that is similar to frame score 6-7 to attain their genetic physiological and biological maturity (Nichols et al., 2001). Depending on the spread between choice and select grades, a decision can be made to look at the risk:benefit of marketing at a lower quality grade. Empty body fat (EBF) for Standard, Select, Low Choice, and Mid Choice Quality grades are 21.1%, 26.2%, 28.6%, and 29.9%, respectively (Nichols et al., 2002).

Table 2. Relationships between Steer Frame Score and Body Weight to Reach 28% EBF<sup>a</sup>

Frame Score	1	2	3	4	5	6	7	8	9
BW, lbs	882	954	1029	1102	1175	1250	1322	1395	1470

<sup>a</sup>Adapted from Nichols et al.(1999)

Finished Holstein steers are typically frame score 8 or 9. Perry et al. (1991) found that when fed to the same degree of marbling, frame score 8 Holstein steers were not different in ADG or DOF but utilized their feed 7% more efficiently when compared to heavier frame score 9 steers. This reduced feed cost of gain. They recommended that Holstein calves with smaller frames should be selected for long-fed Holstein feeding systems and implanted accordingly with expectations of increasing the final market weight by 50 to 100 lbs to ensure a good percentage will attain a low Choice grade.

#### *Protein and implant interrelationships:*

Higher protein requirements for lean tissue gain with larger frame cattle implanted with medium or high potency implants are now suggested based on the emphasis on metabolizable protein which requires different protein levels to optimize performance without increasing the feed cost of gain (Chester-Jones et al., 1998). These authors noted that for implanted Holstein steers fed high energy diets with an ionophore, the lbs of protein required/day increase with faster rates of gain or at heavier BW. The proportion of crude protein (CP) in the diet for a given gain decreases as heavier cattle eat more.

A review by DiCostanzo (1995) evaluated 54 studies using steers between 770 and 1245 lbs fed for 110 to 170 days on feed to describe the relationships between use of medium and high potency implants to dietary protein concentrations on feedlot performance. It was found that CP concentration affected feedlot performance independent of implant strategy. Crude protein intake (CPI) was highest for steers fed high dietary CP regardless of implant strategy. Steer CPI was highest for those implanted with medium or high potency implants and lowest for non-implanted steers regardless of CP dietary concentration. It was noted that the benefit of feeding higher CP diets depends on choice of implants and steer performance response. For maximum performance it was suggested that a high potency terminal implant and 13.3% CP were required. Average daily gain increased 0.13, 0.14, and 0.15 lbs/lb DMI for non-implanted, medium and high potency implanted steers, respectively. Average daily gain increased 0.10 lbs for every percentage increase in dietary CP. It was

shown that implanting yearling feedlot steers with high or medium potency implants required less CP/gain ( 0.73 and 0.76 lb CP/lb gain, respectively) compared to non-implanted steers (0.83 lb CP/lb gain). DiCostanzo (1995) observed that urea was an effective supplemental source fed at < 1% diet DM in diets fed to steers implanted with TBA-based products.

A number of studies at Iowa State University have focused on protein feeding strategies (Trenkle 1995; Lima et al., 1995;Trenkle, 2002) with or without medium or high potency implants. Work has indicated that yearling or young steers implanted with TBA and E have greater protein needs. Charolais x Simmental steers were fed high concentrate diets containing urea (0.85% diet DM; 10.5% CP diet DM) or soybean meal (SBM; 10% diet DM; 13.5% CP diet DM) with monensin sodium (14 mg/lb DM) with or without Revalor-S® implants (implanted on day-1 and re-implanted day-70). Cattle were fed for 183 or 204 days. The greatest economic benefits came from average gain advantages over 141 days for implanted steers. During the first 70 days steers fed SBM gained 27% faster and were 20% more efficient than those fed urea, but by the end of the study there were no effects of CP supplement. Feeding SBM vs. urea increased carcass weight (CW) 21 lbs in implanted steers. Percentage choice decreased from 62% with non-implanted to 47% for implanted steers over 183 days but no effect after 204 days on feed. Implants increased rib-eye area (REA) 1.9 sq. in. vs. no implants with greatest difference between days 150 and 180. Neither protein source influenced the sensory value of the steaks. Quantity of polyunsaturated fatty acids (PFA) in muscle and fat tissue were not affected by implants.

Programmed protein feeding systems using NRC (1996) guidelines were evaluated for 600 lb Angus steer calves (Trenkle, 2002):

- Program I – diet of 13.5% CP (MP ratio – percent of NRC requirement 0.90) for 84 days then reduced to 11.85% (MP ratio 1.1) implanted with Component E-S on day-1 and Component TE-S day-84 (all steers on each program).
- Program II – diet of 13% CP for 84 days, 11.85% CP from 84-112 days and 11.25% CP (MP ratio, 1.19) to 180 days. Implant the same as I.
- Program III fed 13% CP for 84 days, 11.85% at 84-112 days and 10% CP (MP ratio, 1.07) to 180 days.

Feed/gain was lowest for steers on Program I. No other performance or carcass trait differences were noted. The implications of the study were that amount of protein fed to finishing cattle can be reduced without affecting performance and that requirements for ruminally degraded protein as estimated by NRC (1996) may not need to be met with finishing cattle fed high concentrate diets.

#### OVERVIEW OF SOME IMPLANT STRATEGIES EVALUATED IN BOTH BEEF AND HOLSTEIN STEERS IN CONTROLLED STUDIES

There have been numerous beef cattle studies evaluating systems for implanting and re-implanting cattle. Much less data is available for Holstein steers using the more recent implants available. The discussion below will include selected beef breed information that

can be applied to Holstein steers. Considerations include target market date, phase of production, feeding system (programmed or target feeding, number of feeding times/day, diet composition etc.), cattle age and breed, days in the feedlot, potential for compensatory growth, time of day implants are given, implant sequence, and number of implants. An implant database has been established through the Texas Tech Beef Animal Science Dept. web site ([www.asft.ttu.edu](http://www.asft.ttu.edu)) which has been developed in partnership with Intervet. Summary performance and carcass data are available for many designed implant strategies.

#### *Examples of Holstein Steer Studies:*

Work by Chester-Jones et al. (1992a) evaluated the effect of using low to moderate implant strategy for high-energy fed Holsteins from one week of-age to market weight. All calves received their first implant by 42 days of-age when still housed in individual stalls. Strategies compared single, double or triple implants given within 200 days over the growing and finishing period. The study confirmed that for young calves a single implant may have a longer effective pay out than estimated concurring with results by Schaefer et al. (1986). Steers were marketed when pen averages reached 1100 lbs. Percentage choice grade declined with increasing number of implants. Results are summarized in Table 3.

Table 3. Response to single or multiple implants of Ralgro®(R) vs Synovex® (S) for Holstein steers<sup>a</sup>

Implant Sequence Steer Groups (G)	P1 <sup>b</sup> BW	P1 ADG	P2 <sup>b</sup> BW	P2 ADG	P3 <sup>b</sup> BW	P3 ADG	P4 <sup>b</sup> BW	P4 ADG	Overall ADG	DOF to 1100 lb BW <sup>d</sup>
<i>Initial implant @ 42 d of-age – GA<sup>c</sup></i>	lb	lb	lb	lb	lb	lb	lb	lb	lb <sup>d</sup>	BW <sup>d</sup>
Ralgro (R)	154	1.08	393	2.43	698	2.87	1082	2.32	2.32	426
Synovex (S)–C	148	1.05	393	2.29	708	3.13	1077	2.47	2.41	412
<i>42, 126 d – GB<sup>c</sup></i>										
Ralgro	159	1.09	391	2.33	708	3.09	1066	2.10	2.28	432
SC, Synovex S (SS)	149	1.07	392	2.26	717	3.08	1083	2.44	2.39	385
<i>42, 126, 210 d – GC<sup>c</sup></i>										
R-R-R	138	0.91	390	2.36	717	3.08	1083	2.52	2.43	409
SC-SS-SS	137	0.82	409	2.52	702	3.49	1077	2.77	2.55	385

<sup>a</sup>Adapted from Chester-Jones et al.(1992a);

<sup>b</sup>P1 = period 1, day 42 to 53 in individual stalls – all implanted; P2 = period 2, 157 days on feed, 2<sup>nd</sup> implant given at end of P2; P3 = period 3, 257 days on feed, 3<sup>rd</sup> implant given at 199 days on feed; P4 = period 4, 257 to av 410 days. Steers marketed at pen average of 1100 lbs; all period and end BW were taken after withholding feed and water 16 h.

<sup>c</sup>P2 Daily gain - GB, GA Ralgro vs GC Synovex (P < .05), GB Synovex vs GC Synovex (P < .07).

<sup>d</sup>Overall Daily gain – GC Synovex vs GA, GB Ralgro and GB Synovex (P < .05);

DOF- GC Synovex vs all other groups (P < .05).

In a previous study by Chester-Jones et al. (1991), Holstein steers that had received 3 implants prior to 120 days of projected market weight received either no implant, Synovex S or Synovex S combined with Finaplix. Gain response to the 4<sup>th</sup> implant was an increase of 18% and feed/gain 10% less than non-implanted steers. Days on feed were 17 day less for steers receiving four implants. Carcass quality was decreased compared to non-implanted steers. Feed cost/gain was highest for non-implanted steers. Benefits of performance response and less time on feed may offset reduction in gross return from lower carcass quality. A question was asked in a study by Chester-Jones et al. (1996) if delaying the first implant for high-energy long fed Holstein steers until later in the growing period would achieve a greater response when DMI are higher than multiple implants from 42 days of-age? Steers were implanted with a single Compudose® at 600 lbs and gained 19% faster than non-implanted steers to a pen average market weight of 1270 lbs.

Beckett and Algeo (2002) also investigated the effects of delaying implants during the early and intermediate growing phases for Holstein steers from 343 lbs BW. One group of steers received no implants. Other strategies were: a) Ralgro® day 0, TBA + E day 60, none day 120, and TBA + E day 180; b) None day 0, TBA + E day 60, none day 120, TBA + E day 180; c) None day 0, Ralgro® day 60, Ralgro® day 120, TBA + E day 180; d) None day 0 and day 60, Ralgro® day 120, TBA + E day 180. Daily gains did not differ within implant strategy. Implanted steers gained faster, had heavier final weights, and greater REA compared to non-implanted steers. Percentage Choice carcasses were lower for strategy b (27%) and c (31.6%) compared with non-implanted steers (57.9%). Strategy a (40.5%) and d (52.8%) were not different from other treatments. Less desirable meat tenderness was evident in delayed implant groups compared to non-implanted steers. The authors concluded that performance and carcass quality grade were not adversely affected for a delayed implanting strategy but may decrease meat tenderness compared to earlier implants.

Implanting strategy for 280 lb calf-fed Holsteins from a study in California was summarized by Guerrero (1999). The implant strategy, performance and carcass data are summarized in Table 4. It was concluded that implanting Synovex C® on day 1, Synovex S® on day 98 and a combination implant on day 196 (Synovex S® and Finaplix® in this case) appeared to be the optimum for performance and carcass quality. This agrees with the strategy purported by Pritchard (1993) of increasing implant potency with every subsequent re-implant.

Table 4. Effects of Implant on Performance and Carcass Quality of Calf-fed Holstein Steers Fed Continuous High-Energy Diets<sup>a</sup>

Parameter	NI <sup>b</sup>	CSS <sup>b</sup>	CSSF <sup>b</sup>	CSSS <sup>b</sup>	CSSSF <sup>b</sup>	CSSFSF <sup>b</sup>
Initial BW, lb	278	279	280	282	278	278
Final BW, lb <sup>c</sup>	1111	1130	1143	1136	1136	1153
ADG, lb <sup>d</sup>	2.65	2.93	3.00	2.93	2.95	3.02
DMI, lb <sup>d</sup>	14.7	15.5	15.5	15.5	15.2	15.3
DM/gain, lb <sup>de</sup>	5.56	5.27	5.16	5.30	5.12	5.10
Hot CW, lb <sup>d</sup>	694	708	708	717	706	708
Dressing, %	62.5	62.7	61.9	63.1	62.2	61.4
Choice, %	82.2	81.0	64.3	66.3	79.6	72.2
Yield Grade	2.82	2.84	2.78	2.71	2.71	2.53
Retail yield, %	52.0	51.9	52.2	52.2	52.1	52.8

<sup>a</sup>Adapted from Guerrero (1999), University of California Desert Research and Extension Center using 252 purchased Holstein steers

<sup>b</sup>NI = No Implant; CSS = Synovex C® (SC) on day 1, Synovex S® (SS) on day 98 and 196; CSSF = SC day 1, SS day 98, SS and Finaplix® (SF) on day 196; CSSS = SC day 1, SS days 70, 140, and 210; CSSSF = SC day 1, SS days 70, and 140; SF day 210; CSSFSF = SC day 1, SS days 70, SF days 140 and 210.

<sup>c</sup>Finished weight for harvest was an average shrunk BW of approximately 1113 lb.

<sup>d</sup>NI vs all implants ( $P < .01$ ); Implanted cattle 12% > ADG and 4.6% > DMI than NI.

<sup>e</sup>The use and increased frequency of Finaplix® implants enhanced feed efficiency ( $P < .05$ );

One of the earlier studies which evaluated the effect of using a single TBA (140 mg) and E (28 mg) implant (Revalor®) for finishing steers included breed differences between Holstein steers and beef breeds on feedlot performance, carcass quality and composition (Perry et al., 1991). Holstein (H) steers were compared to Angus (A) or Angus x Simmental (AS) cattle when fed a 85% concentrate diet. One half of the steers were implanted (I) with TBA and E, the others received no implant (NI). Steers were harvested when they achieved adequate marbling to attain a low Choice grade as determined by ultrasound. A summary of the results are shown in Table 5. Compared to NI steers daily gain was increased by 17, 26, and 21% for H, A, and AS, respectively. Implanting increased daily protein and fat accretion by 23%. Holstein steers required more DOF to attain the minimum marbling score. Live BW to reach a low Choice degree of marbling was increased 55 to 99 lbs. The implant was given > 120 days before harvest so did not compromise marbling score or number of steers grading Choice.

Table 5. Effect of a Single Trenbolone Acetate and Estradiol Implant (I) vs No Implant (NI) on Performance and Carcass Composition of Finishing Holstein, Angus, and Angus x Simmental Steers Fed a 0.60 Mcal/lb NE<sub>g</sub> diet<sup>a</sup>

Parameter	Holstein (H)		Angus (A)		A x Simmental (AS)	
	I	NI	I	NI	I	NI
<i>Performance:</i>						
Init. BW, lb <sup>bc1</sup>	576	572	627	642	642	636
Final BW, lb <sup>dc2-4</sup>	1173	1126	1166	1078	1133	1120
DOF <sup>125</sup>	210	226	143	146	123	152
ADG, lb <sup>cl-5</sup>	2.86	2.44	3.76	2.97	3.92	3.23
DM/gain, lb <sup>cl-5</sup>	6.50	7.08	5.26	6.06	5.06	5.78
<i>Carcass</i>						
<i>Composition:</i>						
Marbling score <sup>e</sup>	5.0	5.5	5.4	5.4	5.4	5.6
Dressing %	61.8	62.1	62.4	62.8	61.2	62.2
Hot CW, lb <sup>bc2-4</sup>	724	693	717	673	700	693
Conformation <sup>fc1</sup>	6.2	6.3	8.9	8.6	9.1	8.3
REA, sq in <sup>cl</sup>	11.32	10.93	12.03	11.32	12.41	12.32
Backfat, in <sup>cl</sup>	0.29	0.24	0.51	0.43	0.36	0.34
<i>Body</i>						
<i>Composition:</i>						
Carcass fat, %	30.2	30.7	32.7	32.8	31.1	29.8
Carcass prot. %	15.2	14.5	14.4	14.1	14.8	14.9
Prot. gain, lb/d <sup>cl-5</sup>	0.53	0.44	0.64	0.51	0.73	0.59
Fat gain, lb/d <sup>cl245</sup>	0.97	0.88	1.43	1.17	1.41	1.06

<sup>a</sup>Adapted from Perry et al.(1991)

<sup>b</sup>Initial BW of Holstein steers < others (P <.01);

<sup>c</sup>Contrasts P <.01 or P <.05; 1 = H vs others; 2 = I vs NI; 3 = HI vs HNI; 4 = AI vs ANI; 5 = ASI vs ASNI;

<sup>d</sup>Final BW - steers were harvested when ultrasound attenuation indic marbling required for low Choice grade. Final BW were adjusted by dividing hot CW by overall dressing percentage 61.7%.

<sup>e</sup>Marbling score: 4 = slight; 5 = small;

<sup>f</sup>Conformation score: 6 = Good<sup>+</sup>; 7 = Choice<sup>0</sup>; 8 = Choice<sup>+</sup>;

Work by Ainslie et al. (1992) found that Holstein steers implanted with Ralgro® at 350 lbs and fed dry corn and SBM diets containing 7, 22, or 40 % of diet DM as alfalfa silage had higher ADG and lower Feed/Gain over a 98 day growing period compared to non-implanted steers. Steer performance decreased with increasing alfalfa silage levels mainly due to lower DMI. In this study at 98 days, all steers were switched to a 90% concentrate finishing diet and half of the steers were implanted with Revalor®. Steers were harvested when they reached a low Choice marbling score as determined by ultrasound. Implanted steers gained

18% faster and 11% better feed efficiencies compared to non-implanted steers during the finishing period. Overall implanted steers reached their finished BW 37 days earlier than non-implanted steers. Diet and implant program did not consistently affect carcass traits. Level of alfalfa silage in the growing diet did not influence the days to reach finished weights when fed a high-energy finishing diet.

#### *Implants evaluated for pasture-based Holstein steers:*

The evaluation of managing Holstein steers in an intensive rotational grazing system was conducted at the University of Wisconsin Lancaster Research Station from 1995 through 1997 as reported by Chester-Jones et al. (1998). Steers were purchased from a high grain feeding system or off Southern States pastures. Systems evaluated singly or in combination were: a) Unsupplemented or implant control; b) Synovex S® on day 1 and day 84 of the grazing period; c) Daily feeding of 200 mg lasalocid (Bovatec®) in 1 lb of a pelleted wheat middlings supplement (B) and, d) Supplement of coarsely ground corn up to 1% BW. When steers received the SB combination, the corn supplement and Bovatec® equalled 1% of BW. Pasture quality and availability varied across the years. Energy but not protein tended to be a limiting factor in growth rates. Corn supplementation increased BW gains in all years but was relatively inefficient conversion to weight gain. Cost of gains tended to be lower when corn was supplemented in combination with an implant or ionophore. Corn supplementation was beneficial particularly when pasture was limiting. Synovex S® improved daily gains in each year but they exert significant advantage when fat deposition is possible which did not occur in the 380 to 490 lb steers until year 3 when control steers gained over 2.3 lbs daily indicating good pasture availability. In this year the response to an implant investment of \$5 was a gain yield due to implanting of \$34.30. Comerford et al. (2001) used 400 lbs Holstein steers assigned to either 4.5 month grazing then 80% concentrate diets in a feedlot until harvest; 4.5 month grazing with access to molasses-based liquid supplement then similar high corn diets in the feedlot; or placed in the feedlot for the entire feeding period. Half the steers in each treatment were singly implanted with Revalor S®. Implanting steers did not affect carcass traits, carcass composition or finishing phase performance in the feedlot. Implanted steers had faster ADG. Supplementing or unsupplementing pasture-fed Holstein steers in the growing period decreased quality grades and carcass BW when compared to cattle fed throughout in the feedlot.

#### *Example Recent Beef Steer Studies:*

Mader et al. (1999) found that Revalor G® was an effective initial implant for 715 lb cross-bred steers re-implanted with Revalor S® after 66 days vs. initial Synovex S® and Revalor S® as re-implant; initial Ralgro® and Revalor S® as re-implant; single Revalor S® at day zero or day 66 vs. no implants. Non-implanted steers had lowest gains, poorest feed efficiencies, smallest CW, smallest REA but tendency for highest marbling score. Trenkle (1997) found that Synovex Plus® is an effective single implant for finishing steers when compared to those implanted with Synovex S®, Revalor S® vs. no implants. Steers fed Cattlyst® (laidlomycin propionate) in combination with Synovex Plus® showed an improved feedlot performance compared steers fed Cattlyst® alone. In a 145-day finishing study by Cooper et al. (1999), yearling steers were implanted with either Synovex C® on

day 0 followed by Synovex Plus® on day 70; Synovex S® on day 0 and day 70; Ralgro® on day 0 and Synovex Plus® on day 70; and Synovex Plus® on day 0 only. Steers were fed a 13.5% CP diet based on 63% dry rolled-corn, 22.55% wet gluten feed, 7.5% alfalfa hay, 3% tallow and supplement with Rumensin (29 g/ton) and Tylan (10 g/ton). Steers implanted with Synovex C® and re-implanted with Synovex Plus® had better feed efficiencies compared to those implanted with a single Synovex Plus®. Implant strategy did not affect percentage choice carcasses or marbling scores.

Timing of re-implantation has been shown to be as, if not more, critical than product selection. Anderson (2001) reported on a 161 day feeding period with finishing steers twice implanted with either, a) Ralgro® 1<sup>st</sup> - Component TE-S® 2<sup>nd</sup> (re-implanted day 50); b) Component E-C® 1<sup>st</sup> - Component TE-S® 2<sup>nd</sup> (re-implanted day 50) or c) Component E-C® 1<sup>st</sup> - Component TE-S® 2<sup>nd</sup> (re-implanted day 80). Feedlot performance was similar but percentage choice was highest with the 80-day re-implant period. A study by Stanton et al. (1998) with 574 lb black-baldy steers implanted with Revalor S® or Implus S® on day 0. All steers were re-implanted on day 83 with the same type of implants but switched so Revalor S® 1<sup>st</sup> - Implus S® 2<sup>nd</sup> and Implus S® 1<sup>st</sup> - Revalor S® 2<sup>nd</sup>. Steers were re-implanted either at 9 a.m. or 3 p.m. within each group. Feed efficiency was better for a.m. implanted steers compared to p.m.

An example of a strategy for 550 lb medium to large framed steers given by Griffin and Mader (1997) well illustrated a system for back calculating an implant program from the projected finished harvest date. The steers entered the feedlot October 1<sup>st</sup>. The initial implant suggested was estrogenic product such as Magnum, Synovex S®, Implus S®, or Component E-S®. In this example, the market BW was predicted to be 1100 lbs at 3 lbs/day gain with finished date of first two weeks in April. With the 120 day pay out for combination TBA and E from the middle of April would mean a 2<sup>nd</sup> implant the middle to late December ( e.g., Revalor S®, Synovex Plus®, Component TE-S®). Alternatively could use a single Compudose® as the initial implant. Examples of the application of a rationale for implanting beef cattle as outlined by Pritchard (1993) adapted to include the current implants on the market. This is based on the premise of increasing potency with each re-implant although the gain response to the number of re-implants is often decreased:

*Midwestern calf feeding program of 120 days on corn silage and 120 days on a finishing diet:*

- Initial implant lower potency - e.g., Ralgro®, Synovex-C®, Calfoid, Component E-C®,
- 2<sup>nd</sup> Implant after 60 days - e.g., Synovex S®, Implus S®, Revalor IS®, Component E-S®;
- 3<sup>rd</sup> Implant after 90 days – terminal e.g., Synovex Plus®, Revalor S®, Revalor 200®, Component TE-S®; (window 90 to 100 days to harvest)
- Alternative option – use Compudose® as a terminal implant at 150 days.

(Note from the above author - could omit initial implant in longer fed cattle but added early weight gain response is beneficial )

*A 200 day feeding period:*

- Initial implant moderate potency - followed by a higher potency terminal implant at 100 days. (Note from the above author – resorting to 3 moderate potency implants at 70 day intervals can lead to more riding and bullers, especially during last 60 days on feed)

*A 100 to 150 day feeding period:*

- Two moderate potency implants or 1<sup>st</sup> implant of low to moderate potency followed by a terminal implant

*A 60 to 80 day feeding period:*

- Lower potency implant recommended as cattle implant is unknown but moderate potency will lower cost of gain but may more negatively effect carcass quality.

### APPLICATION OF IMPLANT PROGRAMS TO HOLSTEIN STEER FEEDING SYSTEMS

A first consideration for Holstein feeders is to define their market niche whether it be sale of feeders or finished cattle. A next step might be to evaluate the facility options available and calculate the capacity and potential turn around time for varying cattle end points. Make sure that feed resources are available to support predicted performance levels and goals. The next concern is selection of healthy uniform calf or steer groups. Implant strategy should be designed to optimize economic return at each phase of production. Strategies can be dynamic to meet changing market conditions. For finished Holstein steers the strategy will be determined by not only the live market price but also the spread between select and choice grades.

The industry has shown a number of changes over the last 15 years in the acceptance of finished market weights. In the late 1980's continuous high energy-fed Holsteins were acceptable in the market place with final weights of between 1100 and 1200 lbs. Traditionally above 1000 to 1100 lbs Holstein steers will show a marked decline in feed/gain. In a 2-year study reported by Chester-Jones et al. (1992b), 'green' 612 lbs Holstein steers were fed 78% high moisture corn and 18% corn silage based diets to 1000, 1125, 1250, or 1375 lbs pen average market BW. All steers were implanted and re-implanted with Ralgro®. Steers fed to 1255 lbs provided the best return at the time. As the use of higher potency implants became more prevalent the acceptable BW of market Holstein steers increased to offset a perception that carcass quality was being compromised by overuse of high potency implants to maximize feedlot performance. The limits currently appear to relate to market price, carcass size, conformation, and age as feeding options for Holstein steers will ultimately affect the final market weight for the best return. The young Holstein steer can be pushed with high energy diets to take advantage of their genetic potential for efficient growth characteristics to feeder BW of 300 to 450 lbs. With good management, continuous high concentrate feeding programs work well. An alternative is a two-phase feeding system with a growing period of higher forage:concentrate ratio followed by a higher energy diet. An example based on 20 years of Holstein beef research at the Southern Research and Outreach Center might be 3 to 4 parts corn silage:1 part corn from 350 to 700-800 lbs followed by a finishing diet of equal parts of corn silage:corn (Chester-

Jones et al., 1998). A third system is programmed feeding growing and finishing TMR diets in larger feedlots whereby in some instances, pasture raised feeder Holstein steers are moved into the feedlot at 575 to 675 lbs and transitioned to a finishing diet of 0.63 to 0.64 Mcal NE<sub>g</sub>/lb which optimizes performance and net return. These systems will work for lighter feeder steers transitioned through a growing to finishing program (Chester-Jones et al., 1998).

What implant strategies should be used given the above scenarios? Loy (2001) noted that “when developing an implant strategy, the most important implant in terms of improving performance and reducing cost is the last implant used prior to marketing”. This applies to projected harvest date or the sale of light or heavy feeders. Implant strategies for Holstein steers have been proposed by Anderson and Chester-Jones (1991), Siemens, (1996) and Chester-Jones et al.(1998).

Proposed implant strategies – use Table 1 as the basis to select implant products:

*General Strategy examples:*

- Delay lower potency 1<sup>st</sup> implant for long fed Holsteins to 200-300 lb BW if feed intake and growth is optimum; moderate potency 2<sup>nd</sup> implant at 500-600 lbs and higher potency TBA + E combination implant at 95-100 days prior to harvest. If the economics of feed cost/gain allow for > 100 days prior to harvest for a higher potency implant it may be a beneficial safeguard. An alternative proposed is to use a single Encore® implant for calves over 45 days of age for at least a 300 day pay-out if handling facilities are limited or use a Compudose® at 500-600 lb as the final implant.

Note – TBA + E implants should not be used in high silage and moderate growth rate finishing diets containing less than 80% concentrate. Estrogenic based implants should be used as TBA combination implants respond the best when steers are fed high concentrate diets. If cattle are not eating well due to environmental stressors, acidosis or other reasons the final implant is suggested to be an estrogenic based product. If there is a lot of size variation within a pen group that will be marketed over a period of time then a final implant of a moderate potency estrogenic or TBA + E combination (see Table 1) product may be preferable.

- Light feeder steer production from pre-weaning to sale at 400 to 450 lbs – potential of 120-140 days on feed- Implant when steers are > 45 days of-age; Lower potency product and possibility of a 2<sup>nd</sup> implant of moderate potency after 70 days to maintain growth response. Single implant may be adequate as DMI is rapidly increasing post weaning accounting for much of the growth response. Use of two implants from 400-500 lbs to market weight is suggested.
- Heavier Holstein feeder steers from 700-800 to market weight or 100-150 days on feed. Depending on the projected market date and arrangements, implant strategy can be similar to that described above for beef cattle. Two moderate potency implants or 1<sup>st</sup>

implant of low to moderate potency followed by a terminal higher potency implant 95-100 days or > before market.

- Short-fed 900 to 1000+ lbs finishing steers – 60 to 80 days on feed. Often these steers will be large framed from being fed pasture or higher roughage-based diets and will have the potential for compensatory growth when transitioned to high-energy finishing diets. The strategy proposed for beef cattle above is recommended as implant history is unknown. Lower potency is preferable but a moderate potency will lower cost of gain and reduce the time in the feedlot. The compromise may be more negative effect on carcass quality.

## SUMMARY

The availability of single or combinations of estrogenic and androgenic implants to the Holstein steer feeder allows for refinement of implant strategies under varying feeding systems to meet the market and economic return goals as dictated by market price fluctuations. A number of options have been described to illustrate the potential risk:benefits of using implants for growing and finishing cattle. Implant strategies can be readily changed and refined if good record keeping is used to help identify weaknesses of a specific system to be modified.

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# RECEIVING DIETS FOR FEEDLOT CATTLE

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## INTRODUCTION

Nutritional programs for newly arrived feedlot cattle are complicated by the large variation in calf age, pre-weaning diets, and pre-weaning management protocols that exist in the beef cattle industry. This paper will focus on the newly weaned calf, due to the unique situation these animals present compared with yearling animals entering the feedlot. Newly weaned feedlot cattle may be subjected to a considerable amount of stress and multiple stressors immediately prior to and during the marketing process, as well as upon arrival at the feedlot. Perhaps the greatest stress imposed by marketing of calves is weaning. With mammals, weaning is a normal process in the transition to adulthood; however, in beef production, the newly weaned calf is denied both its dam's milk and social contact with her and other adults (Stookey et al., 1997). The result is a period of prolonged vocalization in response to these distresses, which may irritate the respiratory tract and increase susceptibility to infection. Furthermore, when newly weaned calves arrive at the feedlot, they have several nutritional, behavioral, and immunological stressors to overcome. These stressors often include exposure to pathogenic organisms, and vaccines to which the calves must mount an immune response. Additionally, there is often no social order immediately following arrival at the feedlot. The result of this lack of social order is that there are often no trainer animals to demonstrate to newly arrived calves how to eat from a feed bunk or drink from an automatic waterer or water trough. The physical characteristics of the feedlot receiving diet are often drier than pasture or contain fermented feeds unfamiliar to the newly arrived calves. Finally, the calves are often exhausted following weaning, being trucked to a sale barn, sorted and mixed with unfamiliar calves, loaded back onto another truck, and trucked to a feedlot. During this time both feed and water may be scarce. Therefore, during the first two weeks following feedlot arrival, the receiving diet must be both palatable and contain high concentrations of energy, protein, vitamins and minerals in order to allow for proper animal health and growth, and water must be both readily available and kept fresh.

Dietary factors to consider when formulating diets for newly arrived feedlot calves include the expected dry matter intake (DMI), forage to concentrate ratio, energy density, supplemental protein concentration and source, and digestibility of dietary components. Management factors to consider include the cost and availability of the desired feedstuffs, age and weight of the calves, previous exposure to creep feed, desired rate of gain, and whether the calves are going to a forage or silage-based growing program or being placed directly on a high-concentrate finishing diet following the receiving period. Finally, the impact that animal health and ruminal volatile fatty acid (VFA) production have on intramuscular fat deposition may impact the

receiving diet used for calves being marketed live compared with those being marketed on a grid basis.

## GROWTH AND DEVELOPMENT OF RUMINANTS

All nutrients (energy, protein, vitamins, minerals, and water) are used in a hierarchy that goes from maintenance → development → growth → lactation → reproduction → fattening. This means that an animal must have sufficient nutrients to maintain its body before development of an immune response, lung tissue repair, or lean tissue growth can occur, and these must occur before fattening can occur. This hierarchy occurs on a daily basis. Additionally, in general, tissues are deposited in the order of: 1. brain, 2. bone, 3. muscle, and 4. fat. A young, rapidly growing animal that is in a linear phase of growth will naturally put on more bone and muscle. In feedlot receiving diets, where the effects of stress and often an increased body temperature increase an animal's maintenance requirement, it is exceptionally important to assure that the animal is consuming sufficient energy to be in a positive energy balance.

In recent years, considerable evidence has shown that a large proportion of an animal's maintenance energy requirements can be attributed to the visceral organs, especially the liver and gastrointestinal tract, and appear to be associated with the high rates of protein synthesis in these tissues (Ferrell and Jenkins, 1985). In reviewing several papers, Ferrell and Jenkins (1985) reported that a greater proportion of total protein synthesis occurred in the gastrointestinal tract (19 to 23%) and the liver, kidney and pancreas (16 to 17%) than occurred in striated muscle (24 to 28%). The metabolic activity of visceral organs is a function of both the metabolic activity and size of the organs. The maintenance energy requirements of organs change with the relative weights of the organs and are affected by the level of nutrition (Ferrell et al., 1986). Burrin et al. (1989) fed lambs a high-concentrate diet either at ad libitum intake or at a maintenance level of intake. These authors reported that the O<sub>2</sub> consumption in the portal-drained viscera and liver of the lambs fed at maintenance intake was 37 and 63% lower, respectively, than in the ad libitum fed lambs. These authors concluded that both blood flow and O<sub>2</sub> consumption in the liver and portal-drained viscera were related to the level of nutrition, with effects being greatest in the liver. The authors suggested that blood flow to the liver is regulated to ensure a constant rate of both delivery of nutrients and removal of end products for a given unit of tissue, which is determined by liver mass. In another study, Burrin et al. (1990) reported on the effects of feeding lambs a high-concentrate diet at either maintenance or ad libitum levels. They reported that the absolute weights of the liver, kidney, stomach, small intestines, and large intestines from maintenance fed lambs were approximately 52, 72, 63, 63, and 63%, respectively, of those from lambs offered ad libitum access to feed. These authors concluded that the effect of level of feed intake on changes in the relative contribution of visceral organs to whole-body metabolic rate is primarily a result of differences in organ size rather than changes in the metabolic activity of the organs. Furthermore, Burrin et al. (1992) reported that the level of feed intake affected visceral organ mass without changing the DNA mass of the organ. They concluded that changes in visceral organ mass due to changes in the level of feed intake result from changes in cellular hypertrophy (cell size) rather than changes in cell number. Similarly, Rompala et al. (1988)

reported increased stomach complex and large intestine weights when dietary bulk was added to isoenergetic diets, however, no differences in fasting heat production, oxygen consumption or carbon dioxide production were noted. Differences in intestinal mass, and differences in energy source could have rather large implications in metabolic efficiency and growth. The energy sparing effects of restricted feeding on visceral organs occur primarily through reductions in organ size (Fluharty and McClure, 1997). Fluharty et al. (1999) reported on the effects of feeding a high-concentrate diet that was formulated to result in similar daily energy and protein intakes, and an equivalent ADG compared with lambs grazing alfalfa. The lambs fed the high-concentrate diet had lower intestinal organ weights, and retained more than twice as much N on a daily basis compared with lambs that grazed alfalfa. Therefore, dietary manipulation was used to alter nutrient utilization through altering tissue maintenance costs even though energy and protein intake, and ADG were nearly identical. These reductions in maintenance energy and protein requirements are the basis for the enhanced feed efficiencies achieved with most restricted, or limit-fed, feeding systems. For newly arrived feedlot cattle experiencing low feed intake, these studies show that it may be possible to achieve efficient gains, through feeding a diet that is highly digestible. However, caution must be used to assure that over-consumption of high-grain diets does not occur.

#### RUMINAL FUNCTION OF NEWLY ARRIVED FEEDLOT CALVES

Several factors are involved in the ability of newly weaned calves to adapt to their new diet. The periods of feed and water deprivation which normally occur when cattle are weaned and transported by truck cause changes in the rumen environment. Baldwin (1967) reported that the total number of bacteria in the rumen is reduced to 10 to 25% of normal after a 48 h period of feed and water deprivation. Baldwin (1967) used two techniques to measure the functional capabilities of ruminal microbes following a period of feed and water deprivation. When bacteria digest feed, gas is produced. Ruminal fermentative activity (RFA), an indirect measure of microbial activity, was measured by *in vitro* (in a test tube) gas production in the absence of added substrate (sources of food for the bacteria). This was done by sampling the ruminal contents of animals deprived of feed and water, and measuring the gas production of the bacteria present in the sample. Ruminal fermentative capacity (RFC), a measurement of the capacity of ruminal microorganisms to ferment added substrate (sugar) was also measured by *in vitro* gas production. This was done by adding sugar to the sample of ruminal contents. Based on these techniques, Baldwin (1967) reported that RFA is reduced to zero by a 48 h starvation, and RFC is reduced to 10% of pre-starvation levels. However, the source of added substrate can alter the findings of these *in vitro* measurements of gas production. In a more recent study, Cole and Hutcheson (1981) measured RFA and RFC (with a substrate mixture of 50% corn starch and 50% cellulose) for 5 to 7 days (d) following a 48 hour (h) fast in ruminally fistulated steers. These researchers reported that RFC was reduced by as much as 75% during feed and water deprivation, and that RFA and RFC were still lower than pre-fast values at 5 d post-fast. The decrease in RFC during a 48 h fast was confirmed by Cole and Hutcheson (1985), who reported that RFC was reduced 74% ( $P < .05$ ) during a 48 h fast, but returned to control levels by 7 d post-fast. Galyean et al. (1981) reported that steers fasted for 32 h and transported had reduced total

counts of ruminal bacteria, which returned to pre-experiment levels by 72 h post-fast. Therefore, it has been proposed that reduced RFC is one factor limiting feed intake in previously fasted calves for the first 7 to 14 d following the reintroduction of feed and water (Cole and Hutcheson, 1985). However, Cole (1991) reported that exchanging the ruminal contents of lambs that underwent a 72 h fast with those of non-fasted lambs did not affect dry matter intake (DMI), ruminal fluid pH, RFC, or volatile fatty acid (VFA) concentrations. If RFC was a major limiting factor to feed intake, exchanging the ruminal contents of a fasted lamb with the contents of a non-fasted lamb should have increased the DMI of the fasted lamb. Cole (1991) concluded that factors other than RFC and RFA appear to be responsible for reduced feed intake during the first few weeks of the receiving period.

Fluharty et al. (1994a) conducted an experiment with newly weaned, ruminally fistulated steers to determine the effects of energy density and protein source in receiving diets on in situ dry matter (DM), neutral detergent fiber (NDF), and nitrogen (N) disappearance, concentrations of ruminal bacteria, protozoa, ammonia and pH. Fistulated steers were weaned, transported by truck, and held in a sale barn prior to feedlot arrival. On the day of feedlot arrival (d 0), DMI was 62% of DMI on day 7 post-arrival. In situ dry matter digestibility (DMD) 48 h following feedlot arrival was 58.6% compared with 58.2% prior to weaning. This was an indication that the ruminal microbial population was not inhibited in its ability to digest available substrate immediately following weaning, fasting and trucking. Additionally, there were no differences in 48 h in situ NDF disappearance between a pre-weaning sample and d 0 (58.8 vs 57.8%), respectively. Furthermore, no differences occurred in the concentration of total bacteria, or cellulolytic bacteria due to feed and water deprivation. However, concentration of total protozoa was lower on d 0 than at any other time. The results of this study indicated that viable total and cellulolytic ruminal bacteria concentrations are not drastically reduced by weaning and 24 h fasting stresses as previously reported using the indirect RFA and RFC in vitro gas production measurements. In situ orchardgrass dry matter, and fiber, and soybean meal nitrogen disappearance were not reduced upon the day of arrival at the feedlot, suggesting that the ruminal microbial population is able to effectively digest available substrate immediately following weaning, trucking and 24 h of feed and water deprivation. Furthermore, the changes in digestibility over time seemed to be related to changes in dry matter intake, and ruminal pH associated with diet differences. Fluharty et al. (1996) conducted two experiments to determine the effects of length of feed and water deprivation on ruminal microbes and ruminal characteristics of both newly weaned and feedlot-adapted calves. The treatments were as follows: 1) weaned, but not trucked (0 h), 2) weaned, trucked, and fasted for 48 h (48 h), and 3) weaned, trucked, and fasted for 72 h (72 h). With newly weaned calves, on the day of arrival back at the feedlot after treatment, DMI, ruminal volume, and weight of ruminal contents decreased as length of feed and water deprivation increased. However, on d 4 post-arrival (d 4) there were no longer any differences in DMI, or weight of ruminal contents. On d 0, percent rumen DM in the 48 and 72 h treatment groups was lower than the 0-h treatment group, but there were no differences by d 4. There were no decreases in cellulolytic or total bacterial concentration, nor in the ruminal numbers of cellulolytic or total bacteria due to length of feed and water deprivation on d 0 in either newly weaned or feedlot-adapted calves. In both trials, 48

and 72 h of feed and water deprivation decreased protozoal numbers on d 0 and 4 compared with the 0-h treatment group. These results indicate that a period of feed and water deprivation up to 72 h coupled with 8 h trucking does not reduce the concentration or total numbers of either the viable cellulolytic or total bacteria present in the rumen of newly weaned calves or calves already weaned and adapted to a feedlot diet. Ruminal volume, dry matter, total weight of ruminal contents, and protozoal numbers decrease as duration of the fasting period increases, and is related to a reduction in dry matter intake. Therefore, the poor performance and low dry matter intake of newly arrived feedlot calves is not a result of reduced ruminal bacterial numbers.

## DIET ENERGY AND PROTEIN CONCENTRATIONS

Newly weaned feedlot calves have low feed intakes for several days following feedlot arrival, and metabolite utilization or hormonal secretion may alter the intake pattern. Cole et al. (1988) reported feed and water deprivation for 72 h reduced subsequent feed intake for 4 d or more. These authors reported blood metabolite patterns (insulin, glucose and FFA) were abnormal during realimentation. In subsequent studies, Cole et al. (1993) used glucose loading tests to determine that feed deprivation increases serum glucose, FFA and growth hormone and decreases glucose half-life during realimentation. Propionate loading tests also revealed that previously unfed lambs had greater serum growth hormone, propionate and FFA concentrations during realimentation than fed lambs (Cole and Hallford, 1994). Thus, feed and water deprivation had marked effects on hormonal secretion and metabolite utilization, which could impact feed intake. Cole and Hutcheson (1988) suggested that the post-arrival diet should contain a greater quantity of protein than the prefast diet that cattle consumed before feedlot arrival. Furthermore, ruminal degradability of supplemental protein has been shown to affect performance of calves after feedlot arrival (Eck et al., 1988).

Fluharty et al. (1994b) conducted a feedlot receiving experiment with newly weaned calves that had not been previously exposed to creep feed to determine the effects of energy density and protein source in receiving diets containing approximately 13% crude protein (CP) on steer performance and total tract nutrient digestion. The factors compared were energy density (1.80 vs 1.48 Mcal/kg of NEm, referred to as high-energy and low-energy, respectively) and protein source (ring-dried blood meal, BM vs soybean meal, SBM). Diets containing BM resulted in a 10.6% greater ADG, and a 7.6% improvement in efficiency of feed utilization compared with those containing SBM. High-energy diets resulted in an 8.7% improvement in feed efficiency vs low-energy diets. Dry matter digestibility (DMD), NDF digestibility (NDFD), and CP digestibility (CPD) were exceptionally high during wk 1, when DMI was approximately 1.5 kg/d. Nutrient digestibility declined during wk 2 and 3, as intake increased, before stabilizing between wk 4 and 6. Additionally, DMD, NDFD, and CPD were higher on d 7 than at any other time, indicating that by d 7 ruminal function was not adversely affected. Therefore, the reduced feed intake and growth rate normally seen during the first 2 wk in the feedlot is also not caused by a reduction in diet digestibility. The reason for the high digestibility of the diets during wk 1 could be due to a longer ruminal retention time caused by the low feed intakes. Galyean et al. (1979) reported that steers fed an 84% corn diet had higher ruminal and total tract starch

digestion, total tract DMD and total tract OM digestibility as feed intake decreased from 2.0 to 1.0 times maintenance. Additionally, ruminal liquid dilution rate and outflow rate increased with increasing intake. Zinn and Owens (1983a) also reported that organic matter and ADF digestion increased ( $P < .05$ ) with decreasing feed intake from 2.1 to 1.2% of BW in steers fed an 80% concentrate diet.

Fluharty and Loerch (1995) conducted three experiments to determine the effects of protein concentration and protein source on the performance of newly arrived feedlot steers. In experiment 1, the effects of receiving diet CP concentration (12, 14, 16, or 18%) and source (spray-dried blood meal [SDBM] vs soybean meal [SBM]) on steer performance were determined. During the first week following feedlot arrival, there were linear increases in ADG and feed efficiency with increasing protein concentration. For the entire 28 d trial, there was an increase in feed efficiency with increasing CP concentration, and diets containing SDBM compared with SBM. In experiment 2, the effects of receiving diet CP concentration (11, 14, 17, 20, 23, or 26%) on steer performance was determined. Average daily gain and feed efficiency increased with increasing CP concentration during the first week that calves were in the feedlot. There were quadratic ( $P < .01$ ) responses to CP concentration for final weight, ADG, and feed efficiency, indicating that as DMI increased over time the CP concentration of the diet should be decreased. In experiment 3, the effects of receiving diet protein sources on steer performance were determined. The control diet used SBM as the supplemental CP source, and was formulated to contain 12.5% CP. The other five protein sources were corn gluten meal (CGM), ring-dried blood meal (RDBM), SDBM, fish meal (FM), and SBM. For these five CP sources, diets were formulated to contain 23% CP during wk 1, 17% CP during wk 2, and 12.5% CP during wk 3 and wk 4. During wk 1, control steers had the lowest ADG and feed efficiency, while RDBM and SDBM fed steers had the highest feed efficiency. Increased CP concentrations are needed early in the receiving period, when DMI is low. Based on these experiments, the dietary CP concentration early in the feedlot receiving period must meet the animal's gram protein requirement when dry matter intake is low. A variety of supplemental CP sources worked well in the receiving period, when the CP concentration was adequate.

Fluharty and Loerch (1996) conducted three experiments to determine the effects of energy source and level on performance of newly arrived feedlot calves. In experiment 1, the effects of receiving diet and previous creep feed on calf performance were determined. Diets were composed primarily of either corn silage, corn silage + alfalfa pellets + dry corn, or dry corn + alfalfa pellets. For the 41-d trial, calves fed the corn-silage based diet had greater ADG and feed efficiencies compared with calves fed the other two diets. In experiment 2, a 28-d receiving period was used to determine the effects of 16% CP receiving diets containing 70, 75, 80, or 85% concentrate on performance. There were no differences in ADG or feed efficiency due to dietary concentrate level. In experiment 3, the effects of dietary concentrate and protein levels on performance during a 28 d receiving period were determined. The factors were concentrate level (70 vs 85% concentrate), and protein level (12.5%, 16%, or phase-fed at 23%, 17%, 14%, and 12.5% during wk 1, 2, 3, and 4, respectively). During wk 1, calves fed the 85% concentrate diet had greater DMI, ADG, and feed efficiency compared with 70% concentrate. Calves fed the

16% CP, and phase-fed protein diets had greater DMI, ADG, and feed efficiency compared with calves fed the 12.5% CP diets.

Based on these results, receiving diets containing at least 16% CP, and greater than 70% concentrates are beneficial to calves during the first week after feedlot arrival. Furthermore, corn silage-based receiving diets were used successfully in these receiving programs when it was kept fresh on a daily basis. However, creep-fed calves may benefit from a gradual adjustment to corn silage-based diets. Receiving diets containing 70 to 85% concentrate yielded similar animal performance and health status. Providing sufficient energy and protein to calves early in the receiving period enhances performance during this time period.

The effects of protein source on nitrogen metabolism and animal performance are not the same when low-energy diets are fed compared with feeding high-concentrate diets. When diets with highly fermentable carbohydrates are fed, greater microbial protein synthesis occurs compared with low- or moderate-energy diets such as forage based diets (Merchen et al., 1986). This occurs, because the quality of microbial protein synthesized in the rumen is primarily a function of the amount of organic matter fermented in the rumen (Rohr et al., 1986). Additionally, corn based diets may require some additional non-protein nitrogen, because 58 to 73% of the protein from corn is resistant to ruminal degradation (Zinn and Owens, 1983b), which can drastically alter protein supplementation requirements when corn-based diets are fed. Ludden and Cecava (1995) reported that diets containing 70% cracked corn and formulated to be 12.5% CP had metabolizable amino acid flows to the duodenum that were not different between SBM, a high-ruminal escape SBM (SoyPLUS7, SoyPLUS West Central Cooperative, Ralston, IA), or a 50:50 (CP basis) combination of CGM:BM, even when the supplemental protein provided 40% of total dietary CP. As the ruminal N degradability decreased, ruminal microbial protein flow decreased, which negated any increase in duodenal flow of the supplemental protein. Ludden and Cecava (1995) concluded that corn-based diets may be limiting in ruminally degradable protein, especially when the diets are supplemented with high ruminal escape protein, and speculated that SBM resulted in an increase in the production of peptides that were readily utilized by the ruminal microbial population to stimulate microbial growth compared with more ruminally unavailable sources of protein. Furthermore, the pH of the rumen can affect the solubility of a protein source. Soybean meal is less soluble in the rumen of an animal being fed a high-concentrate diet, that results in a lower rumen pH compared with when it is fed as part of a forage-based diet that results in a more basic rumen pH (Loerch et al., 1983). The difference in solubility occurs because proteins are least soluble at their isoelectric pH due to a lack of a net charge, which reduces the electrostatic repulsions between neighboring protein molecules (Clark, 1975). This explains why Fluharty and Loerch (1995) found SBM to be just as effective a source of protein in high-concentrate receiving diets as protein sources normally considered to be higher in rumen bypass.

## NUTRITION, HEALTH, AND CARCASS QUALITY GRADE

A thorough review on the effects of energy and protein on immunity and health of calves has been written previously by Galyean et al. (1999). However, individual health status of calves at

weaning can alter carcass characteristics, and having animals individually identified and being able to track individual animal differences can result in huge differences in carcass value. Gardner et al. (1999) reported that lung lesions from respiratory disease at weaning were present in 33% of their steers at harvest. Lung lesions were present in 37% of steers that had been treated with antibiotics after weaning and 29% of steers never diagnosed with respiratory disease. Steers with lung lesions had lower average daily gains, lighter carcass weights, deposited less internal fat and marbling, and had less tender steaks than animals without lung lesions. This emphasizes the need to carefully monitor individual cattle, and to expect different results at harvest. Furthermore, research at MARC (Wittum et al., 1996) found that 35% of 469 steers in one study were treated for a respiratory disease episode between birth and harvest. In their study, 78% of treated cattle had lung lesions at harvest, and 68% of untreated cattle had lung lesions at harvest. While both groups had high percentages of lung lesions, the authors concluded that if an animal was sick enough to be identified as having a respiratory illness and treated, performance reducing lung damage had already occurred. If a calf gets a respiratory disease, tissue damage occurs, and nutrients are diverted from lean growth and marbling toward repair of the damaged tissue. Additionally, metabolic changes due to the disease increase maintenance energy requirements of the diseased animal, reducing energy availability for growth and fattening. This explains why increasing the concentrate level of feedlot receiving diets above 50%, and altering the protein concentration to meet the animal's requirement for a given level of gain improved average daily gain and feed efficiency with newly weaned calves (Fluharty and Loerch 1995, 1996; Fluharty et al., 1996).

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# CORN PROCESSING FOR FEEDLOT CATTLE

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Corn is commonly fed to feedlot cattle to increase production efficiency. In most feedlots, corn is processed to increase extent of starch digestion. The goal of processing is to make starch more available thereby improving efficiency (Hutchinson, 1997). Multiple methods are available to increase the extent of starch digestion. These include: dry-rolled or cracked corn (DRC), early harvest, ensiled high moisture corn (HMC), steam-flaked corn (SFC), finely ground dry corn (FGC), and reconstituted corn. These are not the only methods available, but are the most common and the methods discussed here. Corn can be fed whole and unprocessed and will be referred to periodically as a reference point. When fed as whole corn, we rely on the feedlot steer to process the corn through mastication.

Numerous reviews are available on starch digestion and corn processing (Theurer, 1986; Huntington, 1997; Owens et al., 1997). Therefore, this paper will briefly review previous publications on energy values and then focus on new concepts relative to corn processing such as: interaction with protein requirements, interaction with byproduct feeding, and how hybrid differences may interact with corn processing.

## PERFORMANCE (ENERGY) COMPARISONS

Few studies are available that directly compare performance for all processing types (whole, HMC, DRC, SFC, FGC). In Owens et al., (1997) review, data were compiled from many feeding trials. The data summary included 183 with DRC, 117 with HMC, and 53 trials with SFC. For all corn feeding, approximately 16,228 head were included from university research reports including journals. Based on feed conversions, HMC diets were 102% the energy of DRC diets, while SFC diet comparisons suggested 112% the value of DRC. In this compilation, whole corn was actually better than DRC and HMC. Some challenges were noted by the authors that may lead to whole corn feeding resulting in better performance such as: 1) unequal roughages across the corn comparisons (7.9, 7.0, 9.3, and 6.0% for DRC, HMC, SFC, and whole corn, respectively), 2) whole corn is processed more by the animal during mastication and rumination, 3) a positive associative effect (or less negative associative effect with whole corn), and 4) shift in site of digestion from rumen to small intestine. Logically, whole corn should not contain more energy than DRC and HMC if processing effectively increases starch digestion.

## SITE OF DIGESTION

Cooper et al., (2002a) fed DRC, HMC, and SFC based diets to finishing steers within the same experiment. Feed efficiency data suggest HMC based diets contained 102% the energy as DRC based diets, whereas SFC based diets contained 113% the energy of DRC based diets. This was a study evaluating degradable intake protein (DIP) requirements and only the 3 high levels (1.0, 1.5, and 2.0% of DM) of urea were compared. Because corn comprises only around 80% of the diet in most of these data, the energy of the corn fraction is even greater than 2% or 12% of DRC

for HMC and SFC, respectively. Comparing Cooper et al., (2002a) to Owens et al., (1997) review, there is excellent agreement for comparisons of HMC and SFC to DRC.

As stated before, the goal of processing corn is to increase the extent of starch digestion. Processing corn would be expected to increase energy use by microbes in the rumen because corn processing increases ruminal starch digestion. Galyean et al. (1976) measured ruminal starch digestibilities of 78, 89, and 83% for DRC, HMC, and SFC based diets, respectively. Cooper et al., (2002b) found starch digestibilities of 76.2, 91.7, and 89.6% for DRC, HMC, and SFC, respectively. Huntington (1997) reviewed 14 experiments from 1986 to 1995 evaluating corn processing influence on ruminal starch digestion and concluded that digestibilities were 76.2, 89.9, and 84.8% for DRC, HMC, and SFC, respectively. These data are consistent with measured starch digestion. Interestingly, HMC is consistently higher for starch digestion in the rumen than SFC, yet SFC results in more efficient feedlot performance.

One consequence of increasing starch digestion in the rumen is the increased risk of ruminal acidosis (Stock and Britton, 1993). The goal is to increase the extent of starch digestion. Unfortunately, processing also increases rate of digestion in the rumen. Presumably, increasing starch digestion in the small intestine would be more energetically efficient (Owens et al., 1986). However, no processing technique is available that increases digestion in the small intestine without increasing rate and extent of ruminal digestion. Therefore, most data suggest that enhancing ruminal starch digestion enhances performance, if acidosis is managed. Starch digestion at the small intestine has limits in ruminants as well (Krehbiel et al., 1996; Kreikemeier and Harmon, 1995).

### CORN PROCESSING INTERACTION WITH PROTEIN REQUIREMENTS

Another consequence of increasing ruminal starch digestion by corn processing is the influence on degradable intake protein (DIP) requirement. Increasing TDN, particularly ruminally digested OM, will increase the DIP requirement (NRC, 1996). Microbial protein production is limited by either protein (DIP) or energy available to microbes. Nutritionally, diet formulation practices should match DIP needs to energy use by microbes. If DIP is limited, starch digestion may be limited as well as subsequent animal performance and microbial supply. Surprisingly, few data are available on true DIP requirements.

Therefore, Shain et al., (1998) evaluated DIP requirements when feedlot cattle are finished on DRC diets and concluded that the requirement was approximately 6.5% DIP as % of DM or 0.9% urea (Table 1). Milton et al., (1997) concluded that the optimum level of urea in DRC diets was between 0.5 and 1.0% urea, with a calculated breakpoint of 0.9%. Cooper et al., (2002a) evaluated DIP requirements in HMC and SFC based diets (Table 1). As predicted, the DIP requirement in HMC and SFC diets are higher than DRC based diets due to increased ruminal digestion. Based on two studies, the DIP requirement in HMC based diets is approximately 9.0% DIP (% of DM). In SFC diets, the results were variable, but averaged 8.3% DIP. Corn protein degradability needs to be accounted for. Average DIP values for corn protein are 40% DIP (% of CP) for DRC and SFC and 60% DIP (% of CP) for HMC.

**Table 1.** Interaction of corn processing (site of digestion) and degradable intake protein (DIP) requirements (Shain et al., 1998; Cooper et al., 2002a).

<b>Dry-rolled corn (Shain et al., 1998)</b>						
<i>urea level (% of DM)</i>	0.0	0.88	1.34	1.96		
ADG, lb	3.15	3.39	3.30	3.41		
F:G	8.13	7.69	7.81	7.63		
<b>High moisture corn (Cooper et al., 2002)</b>						
<i>urea level (% of DM)</i>	0.0	0.4	0.8	1.2		
ADG, lb	3.74	3.79	4.01	4.07		
F:G	7.23	7.03	6.65	6.54		
<b>Steam flaked corn (Cooper et al., 2002)</b>						
<i>urea level (% of DM)</i>	0.0	0.4	0.8	1.2	1.6	2.0
ADG, lb	3.17	3.83	4.41	4.41	4.45	4.49
F:G	7.15	6.21	5.50	5.50	5.59	5.39

## CORN PROCESSING INTERACTION WITH CORN BYPRODUCTS

Because processing corn increases rate of digestion by microbes, rumen acid production is increased and the risk of acidosis is increased (Stock and Britton, 1993). Feeding wet corn gluten feed (WCGF) helps prevent risk of acidosis with high-grain diets (Krehbiel et al., 1995). Therefore, numerous studies have been conducted at the University of Nebraska to determine if energy values are markedly improved in diets containing WCGF when corn is more intensely processed. Scott et al., (2001) evaluated numerous corn processing techniques (Table 2). Feed conversions were improved as processing intensity increased with both calves and yearlings. Ranking of processing based on feed conversions was whole, DRC, FGC, HMC, and SFC for calves. Relative improvements in F:G for DRC, FGC, HMC, and SFC compared to whole corn were 6.8, 10.1, 11.1, and 12.5%, respectively. When fed to yearlings, whole corn was not included, but response to processing was not as marked as with calves. Feeding fine rolled corn (FRC) and HMC did not significantly improve feed conversion compared to DRC. Macken et al., (2003a) fed DRC, FGC, SFC, and HMC processed as rolled (roller mill) and ground (tub grinder) to calves with all diets containing 25% WCGF. Whole corn was not fed in this study, but processing corn more intensely significantly improved performance. Net energy calculated from performance (Owens et al., 2002 and NRC, 1996) was increased by 4.8, 9.1, 11.0, and 14.9% compared to DRC for FGC, RHMC, GHMC, and SFC, respectively.

Interestingly, HMC appears to be much greater in value when diets contain WCGF compared to previous energy comparisons. Based on higher ruminal starch digestibilities for HMC compared to DRC and SFC, perhaps the response is expected in diets where acidosis is not a challenge. For example, the energy value of HMC in diets comprised of HMC only is lower than when fed in combination with other grains (Stock et al., 1991) or in byproduct diets. In previous reviews, HMC feeding improved efficiency by 2% compared to DRC. However, based on work in diets containing 20 to 35% WCGF, cattle fed HMC are 5 to 10% more efficient than similar diets containing DRC. Our conclusion is that intense processing has tremendous value in diets

containing corn byproducts. Fine grinding may also be possible in wet byproduct diets because “fines” settling in bunks is no longer a concern. With SFC, processing appears to improve digestion in the rumen and small intestine compared to DRC. Degradation in the rumen is less for SFC than HMC (Huntington, 1997; Cooper et al., 2002b) yet performance is markedly better when fed to cattle suggesting marked improvement in intestinal digestion.

**Table 2.** Effect of corn processing when diets contain wet corn gluten feed (Macken et al., 2003a, Scott et al., 2001)

<b>Macken et al., 2003a, 25% WCGF</b>					
<i>processing</i>	<i>DRC</i>	<i>FGC</i>	<i>RHMC</i>	<i>GHMC</i>	<i>SFC</i>
ADG, lb/d	4.23	4.35	4.21	4.24	4.33
F:G	5.49 <sup>a</sup>	5.29 <sup>b</sup>	5.13 <sup>c</sup>	5.05 <sup>c</sup>	4.91 <sup>d</sup>
NEg (corn), Mcal/cwt	70.0	73.4	76.4	77.7	80.4
Fecal starch, %	19.2 <sup>a</sup>	11.8 <sup>b</sup>	10.6 <sup>bc</sup>	8.4 <sup>c</sup>	4.1 <sup>d</sup>
<b>Scott et al., 2001, 32% WCGF with calf-feds</b>					
<i>processing</i>	<i>whole</i>	<i>DRC</i>	<i>FGC</i>	<i>RHMC</i>	<i>SFC</i>
ADG, lb/d	4.18	4.24	4.17	4.15	4.25
F:G	5.92 <sup>a</sup>	5.52 <sup>b</sup>	5.32 <sup>c</sup>	5.26 <sup>cd</sup>	5.18 <sup>d</sup>
<b>Scott et al., 2001, 22% WCGF with yearlings</b>					
<i>processing</i>	—	<i>DRC</i>	<i>FRC</i>	<i>RHMC</i>	<i>SFC</i>
ADG, lb/d		3.98 <sup>a</sup>	3.95 <sup>a</sup>	4.02 <sup>a</sup>	4.22 <sup>b</sup>
F:G		6.09 <sup>ab</sup>	6.15 <sup>a</sup>	5.97 <sup>b</sup>	5.54 <sup>c</sup>

DRC=dry rolled corn, FGC=fine ground corn, FRC=fine rolled corn, RHMC=rolled high moisture corn, GHMC=ground high moisture corn, SFC=steam flaked corn, whole=whole corn

### INTERACTION OF CORN PROCESSING AND HYBRIDS

Corn starch type and physical characteristics can have an impact on feedlot performance and starch utilization. Endosperm (i.e. starch) can be classified based on vitreousness or hardness. Flinty genotype hybrids have more hard endosperm, whereas dent genotype hybrids contain a larger proportion of soft, floury type (less vitreousness) endosperm. Philippeau et al., (1999) evaluated numerous physical characteristics of corn grain differing in endosperm type using in situ digestion techniques with dacron bags. Apparent density and vitreousness of the endosperm were highly correlated to starch and DM in situ degradation (Philippeau et al., 1999). Macken et al., (2003) hypothesized that endosperm type may interact with processing. Two hybrids were chosen representing endosperm types that were as different as commercially available within dent genotypes (common U.S. dent corn) and were termed flinty and floury to represent endosperm vitreousness, not genotype. Hybrids were then fed as either DRC or HMC. Feed conversion was impacted by both endosperm type and processing; however, an interaction was observed for feed conversion (Table 3). Feeding corn as HMC resulted in similar performance between the flinty and floury hybrids. However, when fed as DRC, feed conversion was increased (poorer) for both hybrids, but even more so for the flinty hybrid. Digestibility data suggest that corn hybrids containing more vitreous endosperm are lower in digestibility for both OM and starch.

**Table 3.** Interaction between starch endosperm type and corn processing (Macken et al., 2003b)

Endosperm type <i>processing</i>	Floury		Flinty	
	<i>HMC</i>	<i>DRC</i>	<i>HMC</i>	<i>DRC</i>
feedlot				
ADG, lb/d	3.65 <sup>a</sup>	3.61 <sup>a</sup>	3.61 <sup>a</sup>	3.34 <sup>b</sup>
F:G	5.36 <sup>a</sup>	5.55 <sup>b</sup>	5.37 <sup>a</sup>	5.88 <sup>c</sup>
metabolism				
DMI	20.3	20.5	19.5	18.7
OM digestion, % <sup>c</sup>	86.4	87.3	84.7	85.2
Starch digestion, % <sup>c</sup>	98.1	97.6	95.3	95.6

<sup>c</sup>Main effect of endosperm type, but not processing

### SUMMARY

Considerable progress has been made on evaluating corn processing techniques, particularly interactions with protein requirements, corn byproduct feeding, and a new focus on how processing may interact with hybrid (starch) types. Clearly, processing corn improves performance in most situations. In specific feeding situations, the energy value may be “masked” by too rapid rate of starch digestion and acidosis related challenges. However, if starch digestion is increased in the rumen, DIP requirements are increased. The increase in DIP requirements is predictable and is driven by energy utilization by microbes. Feeding byproducts will alleviate acidosis related challenges, therefore, intense processing such as high moisture corn, fine grinding, and steam flaking will result in improvements in efficiencies of up to 15%. In the future, hybrids may be developed that will result in similar performance when fed as dry rolled corn compared to another corn hybrid fed as processed corn. Nutritional value for feedlot cattle has not been considered in the past for corn starch improvement. However, processing may overcome some of the negative characteristics of starch packaging and corn type. More research will be conducted in this area at the University of Nebraska in the future.

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# USE OF OLIGOSACCHARIDES AND GUT MODIFIERS AS REPLACEMENTS FOR DIETARY ANTIBIOTICS

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## INTRODUCTION

For the past 4 decades, antibiotics have been supplemented to poultry feed to improve the growth performance and protect birds from the adverse effects of pathogenic and non-pathogenic enteric microorganisms. Despite the abundance of scientific data that has been presented on the benefits of antibiotics, there is limited information about how antibiotics promote growth, and more specifically, how they affect the physical attributes of the animal and the microbial populations that reside within the gastrointestinal tract. Antibiotics have come under increasing public scrutiny because of the potential development of antibiotic-resistant human pathogenic bacteria after long use (Phillips, 1999; Ratcliff, 2000). Consequently, the poultry industry must develop alternatives to antibiotic growth promoters and reduce the amount of antibiotics used to maintain efficient poultry production and produce safe poultry meat and egg products. Recently, there has been considerable interest in the use of oligosaccharides, namely mannanoligosaccharide (MOS) and fructooligosaccharides (FOS) as alternatives to antibiotics. This paper will review the mechanisms of antibiotic action to promote growth in poultry and compare it to the effects of dietary supplementation of oligosaccharides.

## ANTIBIOTIC GROWTH PROMOTANTS AND MECHANISMS OF ACTION

Antibiotics have been shown to improve the growth and feed efficiency of broilers (Woodward et al., 1988; Miles et al., 1984) and turkeys (Salmon and Stevens, 1990; Waibel et al., 1991), decrease flock variability (Miles and Harms, 1984), and increase the intestinal digestion and absorption of carbohydrates and fats (Eyssen and De Somer, 1963a,b). There are several theories why antibiotics influence the gut microflora and growth performance of poultry. First, antibiotics control and limit the growth of detrimental microbes (Truscott and Al-Sheikhly, 1977). Second, antibiotics limit the growth and colonization of numerous non-pathogenic species of bacteria in the gut that produce antagonistic metabolites, such as ammonia (Tannock, 1997), which adversely affect the physiology and morphology of the gut (Visek, 1978; Postma et al., 1999). Wostmann et al. (1960) compared penicillin-fed birds to germ-free birds and found that conventional birds consuming the antibiotic treatment had reduced amounts of ileal lamina propria and reticulo-endothelial components almost similar to levels seen in the germ-free birds. A thinner intestinal epithelium in germ-free or antibiotic-fed animals may enhance nutrient absorption (Visek, 1978) and reduce the metabolic demands of the gastrointestinal system. "Thinning" of the gastrointestinal walls tract may be due to the inhibition of the microbial production of polyamines and volatile fatty acids, known to increase enterocyte turnover rate and activity. This increased net energy committed to maintaining the luminal tissue comes at the expense of more productive purposes such as muscle accretion (Bedford, 2000). 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).

Wide use of antibiotic growth promotants in poultry is one reason the public is placing some blame of antibiotic resistance of potential pathogens on the poultry industry. This blame may be partly justifiable. Antibiotic resistance has been displayed by field *Escherichia coli* isolates from commercial turkey farms in North Carolina, including resistance to Enrofloxacin (Fairchild et al., 1998). Although there are no specific claims that growth promoter antibiotics control disease (Gustafson and Bowen, 1997), the debate over resistance seen among Gram-negative bacteria, such as *E. coli* and *Salmonella*, has generated the strongest objection to antibiotic use (Scioli et al., 1983; Gustafson and Bowen, 1997). It has been reported that antibiotic resistance of indigenous *E. coli* of poultry has remained at a relatively high level since the 1950's (Gustafson and Bowen, 1997). Concern about the development of antibiotic resistance among potential human pathogens necessitates the need for more study of alternative growth promotion therapies.

## MANNANOLIGOSACCHARIDES

### Effect on pathogen colonization

The cell wall of the yeast organism consists of carbohydrates and proteins in the form of chained and branched structures of glucose, mannose, and N-acetylglucosamine (Ballou, 1970). 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. Newman (1994) reported that the presence of dietary MOS in the intestinal tract removed pathogenic bacteria that could attach to the lumen of the intestine in this manner. Mannose was shown by Oyofe et al. (1989a) to inhibit the *in vitro* attachment of *Salmonella typhimurium* to intestinal cells of the day old chicken. Then Oyofe et al. (1989b) provided evidence that dietary D-mannose was successful at inhibiting the intestinal colonization of *Salmonella typhimurium* in broilers.

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 (*Saccharomyces cerevisiae*, NCYC 1026). Five of seven strains of *E. coli* and 7 of 10 strains of *Salmonella typhimurium* and *S. enteritidis* agglutinated MOS and *Sac. cerevisiae* cells. However, strains of *S. choleraesuis*, *S. pullorum*, and *Campylobacter* did not lead to agglutination. They also determined the effect of MOS on cecal fermentation parameters, cecal microflora, and enteric pathogen and coliform colonization in chicks. After 3-day old chicks were orally challenged with  $10^4$  cfu of *S. typhimurium* 29E and received 4000 ppm dietary MOS, cecal *S. typhimurium* 29E concentrations decreased from 5.40 to 4.01 log cfu/g ( $P < 0.05$ ) at day 10. A similar study using *S. dublin* as the challenge pathogen resulted in a decrease in the number of positively infected birds by day 10 from 90% to 56% ( $P < 0.05$ ). Dietary MOS supplementation also reduced the concentration of cecal coliforms, although less significantly ( $P < 0.10$ ) as with the *Salmonella* challenges. Dietary MOS supplementation had no effect on cecal concentrations of lactobacilli, enterococci, anaerobic bacteria, lactate, volatile fatty acid, or cecal pH.

The effects of hen age, *Escherichia coli*, and dietary MOS and bambarmycins on poult performance from 1 to 21 d were studied previously by Fairchild et al. (1999). Day-of-hatch BUTA (BIG-6) male poults were gavaged (1 mL) with  $1 \times 10^8$  cfu / mL *E. coli* composed of 4 serotypes or sterile carrier broth. A mixture of the same *E. coli* cultures was added to the drinking water ( $1 \times 10^6$  cfu *E. coli* / mL drinking water) on a weekly basis to ensure a continuous bacterial challenge. Within each *E. coli* split plot treatment group, poults from hens of different ages (33 and 58 wk of age) were fed diets containing 1 kg MOS/ton feed) and 2 g bambarmycins/ton feed, alone and in combination, in a randomized complete block design. One bird per pen (n=128) was randomly chosen at 1 and 3 weeks of age for bacterial sampling of liver and intestinal tissue for coliforms, aerobic bacteria, and *Lactobacillus* spp. Individual

body weights and feed consumption by pen were recorded weekly and poult mortality was recorded daily. *Escherichia coli* isolates from tissue samples were O stereotyped. Under *E. coli* challenge, dietary MOS and bambermycins improved ( $P < 0.05$ ) poult body weight gains. When poult were not challenged with *E. coli*, dietary MOS improved ( $P < 0.05$ ) poult growth during the second week, while dietary bambermycins improved ( $P < 0.05$ ) poult growth through the third week. Cumulative 3-week body weight gains for unchallenged poult were improved ( $P < 0.05$ ) by both MOS and bambermycins. Two of the four *E. coli* stereotypes administered were recovered in cultures of tissue samples. Several stereotypes were recovered that were not administered. This work demonstrates that dietary MOS can improve the overall performance of poult, especially when they are faced with an *E. coli* challenge, as well as traditionally used antibiotics.

#### Dietary MOS versus antibiotics (Virginiamycin and Bambermycin)

Several studies have demonstrated the performance benefits of feeding yeast cultures to growing poultry (Hayat et al., 1993; Bradley, 1994; Bradley and Savage, 1995). Improved performance has also been reported in broilers and turkeys receiving dietary MOS (Shane, 2001). Turkeys are relatively more sensitive to enteric challenges than broilers because of their rapid growth rate and longer growth period. Any enteric challenges that do arise in turkeys are often difficult to treat because of regulatory restrictions or pharmaceutical limitations. Therefore, non-pharmaceutical enteric conditioners, such as MOS, are of particular interest to the turkey industry. The balance of this section will focus on our research with turkeys, some of which can be applied to broiler applications. A series of turkey experiments were conducted at North Carolina State University to compare the effects of antibiotic growth promoters (virginiamycin and bambermycin) and MOS on growth performance and enteric conditions.

The objectives of the first study were to compare the effects of MOS, virginiamycin, bambermycin and their combinations on growth performance, enteric microflora metabolites, and dietary energy and protein utilization. We hypothesized that MOS would promote growth by maintaining a normal stable gut microflora, while the antibiotics would elicit their positive effects by altering microflora fermentation. Hybrid<sup>®</sup> Large White male poult were randomly assigned to 48 pens (9 m<sup>2</sup>/pen) on day of hatch and reared until 140 d while subjecting them to 6 dietary treatments: 1) Control- typical U.S. corn & soybean meal diet; 2) MOS (Bio-Mos<sup>®</sup>, Alltech, Inc., Nicholasville, KY 40356) (1 kg/ton to 6 wk then 0.5 kg/ton); 3) BM (Flavomycin<sup>®</sup>, Hoechst Roussel Vet, Warren, NJ 07059) (2 g/ton active ingredient); 4) VM (Stafac<sup>®</sup>, Pfizer, Inc., Exton, PA 19341) (20 g/ton active ingredient); 5) MOS+BM (MOS and BM dietary inclusion rates as in 2 and 3); and 6) MOS+VM (MOS and VM dietary inclusion rates as in treatments 2 and 4). All diets were pellet-processed and were formulated to meet or exceeded NRC (1994) nutrient recommendations for turkeys.

Growth performance. Significant treatment effects on growth performance were observed (Table 1). Dietary supplementation with MOS, BM, and VM resulted in improved body weight (BW) and feed/gain ratio (F/G). All three dietary supplements improved 20 wk BW. However, there were no additional improvements in 20-wk BW when either of the growth promoters was fed in conjunction with the MOS. Birds fed MOS or VM had significantly increased BW at 20 wk, with MOS-fed birds exhibiting the best market weights. Birds fed BM, VM, and MOS+BM had increased 12 wk BW, but these differences disappeared at 15 wk. All three additives (MOS, BM, and VM) and the combinations MOS+BM and MOS+VM improved F/G for 0-3 wk of age. From 3-6 wk of age, the VM and MOS+BM treatment improved F/G, suggesting a possible synergistic action between the two compounds. MOS and VM also improved F/G from 15-18 wk of age. VM, MB, and MV improved cumulative FG for 0-6, 0-12 wk, and 0-18, but this difference was lost during the last 2 weeks of the trial because of increased variability due to uncontrolled feed wastage as the large birds became more crowded within the pens. There were no significant treatment effects on mortality rate.

Table 1. Effects of mannanoligosaccharides (MOS), bambermycins (BM), and virginiamycin (VM) on the body weights and feed/gain of male turkeys<sup>1</sup>

Treatment	Body Weight (Kg)				Feed/Gain <sup>2</sup>		
	3 wk	12 wk	18 wk	20 wk	0 - 3 wk	0 - 12 wk	0 - 18 wk
Control	.668 <sup>a</sup>	7.84 <sup>b</sup>	15.07 <sup>a</sup>	17.48 <sup>b</sup>	1.49 <sup>a</sup>	2.04 <sup>a</sup>	2.44 <sup>a</sup>
MOS	.668 <sup>a</sup>	7.94 <sup>ab</sup>	15.41 <sup>a</sup>	17.94 <sup>a</sup>	1.43 <sup>b</sup>	2.02 <sup>ab</sup>	2.40 <sup>ab</sup>
BM	.674 <sup>a</sup>	8.12 <sup>a</sup>	15.39 <sup>a</sup>	17.81 <sup>a</sup>	1.41 <sup>b</sup>	2.00 <sup>abc</sup>	2.41 <sup>ab</sup>
VM	.679 <sup>a</sup>	8.17 <sup>a</sup>	15.41 <sup>a</sup>	17.85 <sup>a</sup>	1.40 <sup>b</sup>	1.95 <sup>c</sup>	2.36 <sup>b</sup>
MOS-BM	.664 <sup>a</sup>	8.13 <sup>a</sup>	15.43 <sup>a</sup>	17.80 <sup>a</sup>	1.43 <sup>b</sup>	1.98 <sup>bc</sup>	2.39 <sup>b</sup>
MOS-VM	.673 <sup>a</sup>	8.06 <sup>ab</sup>	15.33 <sup>a</sup>	17.61 <sup>ab</sup>	1.40 <sup>b</sup>	1.97 <sup>bc</sup>	2.36 <sup>b</sup>
SEM (39) <sup>3</sup>	9	0.08	0.13	0.12	0.02	0.02	0.02

<sup>a-b</sup> Means with different superscripts within a column differ significantly ( $P < 0.05$ ). There were no significant differences in poult starting weights at one d of age (60 g).

<sup>1</sup> Values represent means of 8 replicate pens containing 20 birds per pen reduced to 12 birds per pen at 12 wk of age.

<sup>2</sup> Cumulative feed/gain is adjusted for mortality losses.

<sup>3</sup> SEM(39) = Standard error of the mean with 39 degrees of freedom.

**Enteric Characteristics and Gut Fermentation.** At 12 wk of age, three birds per pen were randomly chosen from the control, MOS, BM, and VM treatments to obtain samples for the measurement of the physical characteristics of the gastrointestinal tract and microbial metabolite content in the jejunum and ceca. The duodenum, jejunum, and ileum segments were measured for total weight and length, and then mucosa and muscularis weights from 10 cm sections from each segment were determined. Jejunal and cecal digesta were analyzed for volatile fatty acid (VFA) concentrations (acetic, propionic, and butyric), pH, lactic acid concentrations, and ammonia. Finally, apparent metabolizable energy of the feed (adjusted for nitrogen) ( $AME_N$ ) was determined by analyzing the feed and ileal contents for dry matter content, total nitrogen, and gross energy.

The growth promotion observed among the antibiotic treatments was associated with significant decreases in weights and lengths of intestinal segments. In contrast, MOS had neither significant effects on relative intestinal weights nor differences in mucosa and muscularis tissue mass (Table 2). Virginiamycin-fed turkeys exhibited decreased weights of duodenum, ileum, ceca, and colon as compared to the control-fed birds, but BM had marginal effects on the weights of these gut segments. Other researchers observed similar effects of antibiotics on intestine mass (Dafwang et al. 1985; Stutz et al., 1983; Hill et al., 1957). Henry et al. (1986, 1987) reported a 19% and 14% decrease in intestinal weight in broiler chicks from the dietary inclusion of VM and BM, respectively, primarily due to a visible thinning of the intestinal tract wall. Feeding Penicillin and Aureomycin also has been shown to decrease the intestinal weights of chicks (Pepper et al., 1953; Coates et al., 1955). Antibiotics limit microbial population numbers and their production of toxins and by-products (primarily from Gram-positive bacterial species) in the lumen, they reduce the competition with the host for vital nutrients, and they enhance the absorption and utilization of nutrients due to a thinning of the intestinal wall (Visek, 1978; Waldroup et al., 1970; Caston and Leeson, 1992; Waibel et al., 1991; Buresh et al., 1986). Gordon and Bruckner-Kardoss (1961) stated that the presence of the normal microflora in the lumen of the intestines of conventionally reared animals imparts an inflammatory effect on the cells lining the intestinal wall. After comparing conventional and germ-free chicks as a model to understand the effects of antibiotic growth promotion, Visek (1978) concluded that enteric microflora not only increases intestinal mass (small intestinal weight, lymphoid tissue, and reticuloendothelial cells), but tissue turnover rate increases up to 40% due to the presence of intestinal microbes (Visek, 1978). Indeed, small intestinal tissue is the most rapidly regenerating tissue in the body (LeBlond and Walker, 1956) and it represents a significant metabolic load if the turnover rate may increase for any reason.

Table 2. Effects of mannanoligosaccharides (MOS), bambermycins (BM), and virginiamycin (VM) on the intestinal segment weights and lengths, and wet weights of small intestinal mucosa and muscularis of male turkeys <sup>1</sup>

Gut Segment	Dietary treatment				SEM (25) <sup>2</sup>
	Control	MOS	BM	VM	
	----- Relative Weight (g/kg BW)-----				
Duodenum	3.01 <sup>b</sup>	3.15 <sup>ab</sup>	3.25 <sup>a</sup>	2.63 <sup>c</sup>	0.09
Jejunum	6.88 <sup>a</sup>	7.08 <sup>a</sup>	7.08 <sup>a</sup>	6.51 <sup>a</sup>	0.18
Ileum	6.96 <sup>ab</sup>	7.37 <sup>a</sup>	6.91 <sup>b</sup>	6.26 <sup>c</sup>	0.17
Cecum	4.00 <sup>a</sup>	4.12 <sup>a</sup>	4.08 <sup>a</sup>	3.65 <sup>b</sup>	0.07
Colon	1.46 <sup>a</sup>	1.43 <sup>a</sup>	1.41 <sup>a</sup>	1.30 <sup>b</sup>	0.03
	----- Relative Length (cm/kg BW)-----				
Duodenum	4.22 <sup>a</sup>	4.10 <sup>a</sup>	4.06 <sup>a</sup>	3.69 <sup>b</sup>	0.08
Jejunum	10.22 <sup>a</sup>	10.28 <sup>a</sup>	10.12 <sup>ab</sup>	9.62 <sup>b</sup>	0.17
Ileum	10.54 <sup>a</sup>	10.83 <sup>a</sup>	10.65 <sup>a</sup>	10.14 <sup>a</sup>	0.20
Cecum	3.16 <sup>a</sup>	3.14 <sup>a</sup>	3.34 <sup>a</sup>	3.07 <sup>a</sup>	0.08
Colon	1.21 <sup>a</sup>	1.16 <sup>a</sup>	1.16 <sup>a</sup>	1.14 <sup>a</sup>	0.02
	----- Mucosa Weight (g/kg BW)-----				
Duodenum	0.55 <sup>bc</sup>	0.58 <sup>ab</sup>	0.67 <sup>a</sup>	0.46 <sup>c</sup>	0.04
Jejunum	1.10 <sup>a</sup>	1.10 <sup>a</sup>	0.89 <sup>b</sup>	1.20 <sup>a</sup>	0.07
Ileum	0.81 <sup>a</sup>	0.87 <sup>a</sup>	0.85 <sup>a</sup>	0.76 <sup>a</sup>	0.06
	----- Muscularis Weight (g/kg BW)-----				
Duodenum	1.49 <sup>a</sup>	1.60 <sup>a</sup>	1.62 <sup>a</sup>	1.33 <sup>b</sup>	0.06
Jejunum	2.82 <sup>a</sup>	2.89 <sup>a</sup>	2.71 <sup>ab</sup>	2.52 <sup>b</sup>	0.08
Ileum	2.86 <sup>a</sup>	2.96 <sup>a</sup>	2.87 <sup>a</sup>	2.42 <sup>b</sup>	0.14

<sup>a-c</sup> Means with differing superscripts within a row differ significantly ( $P < 0.05$ ).

<sup>1</sup> Measurements made on a wet weight basis and represent average values of 3 sampled birds per pen from 8 replicate pens per treatment.

<sup>2</sup> SEM (25) = Standard error of the Mean with 25 degrees of freedom.

The effect of VM on intestinal segment weights was more attributed to a decrease in the muscularis rather than mucosa in each segment. Therefore, VM exerted its effects mainly on the underlying circular and longitudinal muscle layers and not the epithelial layer. In contrast to the results of our study, Gordon and Bruckner-Kardoss (1961) reported a greater impact of antibiotics on the lamina propria (mucosa) region than on the underlying muscular region (muscularis). The authors concluded that the decrease in microflora numbers decreased the need for lymphatic tissue in the lamina propria. The BM treatment resulted in increased relative duodenal weight primarily associated with the mucosa, indicating a slight stimulatory effect on duodenal lymphatic tissue. The effect of VM on increased intestinal muscularis is reasonable because it may be associated with a reduced need for gut motility to control microbial activity. An increase in gut motility occurs during gastrointestinal distress, resulting in diarrhea (Vispo and Karasov, 1997). In several animal species, interruptions of the gastrointestinal ecosystem can result in hypertrophy of the muscular layer that is associated with a 3 to 4-fold increase in the contractile strength of the muscle fibers (Johnson, 1994).

In comparison to the well-documented effects of antibiotics, little is known about the effects of dietary MOS on the intestinal tissue of poultry. Dietary MOS had only a marginal stimulatory effect on the physical attributes of the intestines of turkeys. Intestinal lymphoid tissue may be more developed or active due to MOS (Newman, 1994; Cotter, 1997), which may explain the slight thickening of the

intestinal tract observed in MOS-fed turkeys in the current study. However, definitive data are lacking with respect to the effects of MOS on the immune system of male turkeys. The reported positive effects of MOS is attributed to a competitive binding of pathogenic microbes, including *E. coli* and *Salmonella*, thus suppressing their colonization in the gut. Suppression of disease-causing bacterial colonization by MOS may not be associated with a decrease in the stimulation of mucosal lymphatic tissue and enteric motility.

Table 3. Effects of mannanoligosaccharides (MOS), bambermycins (BM), and virginiamycin (VM) on microbial by-product production characteristics in the jejunum and dietary AMEn of male turkeys at 12 weeks of age<sup>1</sup>

Measurement	Dietary Treatment				SEM(25) <sup>2</sup>
	Control	MOS	BM	VM	
pH level	6.63 <sup>bc</sup>	6.52 <sup>c</sup>	6.72 <sup>ab</sup>	6.93 <sup>a</sup>	0.11
	----- (mmol/L) -----				
Ammonia	2.71 <sup>a</sup>	2.16 <sup>a</sup>	2.10 <sup>a</sup>	2.12 <sup>a</sup>	0.36
Lactic acid	4.62 <sup>a</sup>	4.27 <sup>a</sup>	5.64 <sup>a</sup>	4.15 <sup>a</sup>	0.49
Acetic	8.63 <sup>a</sup>	5.59 <sup>a</sup>	7.08 <sup>a</sup>	6.47 <sup>a</sup>	1.05
Propionic	11.70 <sup>a</sup>	6.40 <sup>b</sup>	6.33 <sup>b</sup>	7.24 <sup>b</sup>	1.12
Butyric	2.18 <sup>a</sup>	1.68 <sup>a</sup>	2.20 <sup>a</sup>	1.94 <sup>a</sup>	0.20
Total VFA	21.33 <sup>a</sup>	12.68 <sup>b</sup>	13.26 <sup>b</sup>	15.85 <sup>ab</sup>	2.05
Kcal AMEn / kg diet <sup>3</sup>	2853 <sup>b</sup>	2937 <sup>a</sup>	2847 <sup>b</sup>	2931 <sup>a</sup>	40
% difference: relative to control	-	+ 2.94	- 0.21	+ 2.73	-

<sup>a-b</sup> Means with differing superscripts within a row differ significantly ( $P < 0.05$ ).

<sup>1</sup> Measurements represent average values of 3 sampled birds per pen from 8 replicate pens per treatment.

<sup>2</sup> SEM(25) = Standard error of the mean with 25 degrees of freedom.

<sup>3</sup> Apparent ME corrected for nitrogen retained was calculated using ileal contents.

Even though the ceca are the primary site of gut microflora fermentation, fermentation in the jejunum has a greater influence on digestion and nutrient absorption (Table 3). In comparison to the control treatment, the antibiotics increased jejunal digesta pH (VM more significantly than BM). This increase in pH was associated with a reduction in total VFA concentration of the digesta, mainly attributed to significant reductions in propionic acid, followed by moderate reductions in the other VFAs. Antibiotics, such as clindamycin, bacitracin, and vancomycin, decrease bacterial fermentation as indicated by reduced fecal levels of short chain fatty acids (Cummings, 1995). Dietary MOS also reduced total VFA concentration significantly. In contrast to VM and BM, however, MOS decreased jejunal digesta pH. Although not statistically significant, MOS and the antibiotic treatments also reduced jejunal digesta ammonia content. There were no significant treatment effects on lactic acid concentration.

Fermentation products in the gut can be used to characterize the microflora population. Volatile fatty acids and ammonia are produced from the fermentation of carbohydrates by glucose-fermenting bacteria and proteolytic bacteria (Hudson and Marsh, 1995). Acetic acid is the major product of microflora that use cellulose and other fibrous carbohydrates as their primary substrate. Propionic acid is the major product of microflora that use starches and sugars as their primary substrate. Butyric acid is produced by microflora who prefer proteins as their primary substrate. These VFA's may provide benefits to the host animal, including: 1) use of acetic acid as a metabolic fuel for muscle, kidney, heart, and brain tissue 2) utilization of propionic acid for gluconeogenesis by the liver and 3) use of butyric acid as a major fuel source in the enterocyte (Cummings, 1995). The VFA's also have been shown to possess bacteriostatic and bacteriocidal properties (Barnes et al., 1979, 1980; Corrier et al., 1990) against organisms such as *Salmonella* and *E. coli*. Lactate, produced mainly by saccharolytic bacteria (*Lactobacillus*, *Bifidobacteria*, *Enterococcus*, *Pedococcus*, and *Streptococcus*) during the fermentation of carbohydrates,

may serve to protect the animal from pathogenic bacteria (*Salmonella*, *E. coli*, *Clostridium*) by decreasing the pH of the hindgut, thus impeding the growth of these unfavorable bacteria (Barrow, 1992). Broad-spectrum Gram-positive antibiotics, such as BM and VM would serve to limit the growth of these beneficial microbes and may result in a decrease in intestinal levels of lactic acid.

Enteric conditioners, such as MOS and antibiotic growth promoters, ultimately enhance the efficiency of nutrient utilization by reducing the competition between the host and its intestinal microbial inhabitants. Without the microbial competition for energy and other nutrients, the host retains a greater amount of nutrients available for absorption and metabolism. In our study, dietary inclusion of MOS and VM resulted in over 2.5% better dietary energy utilization (AMEn) than the control-fed or BM-fed turkeys (Table 3). These results agree with the improvements in feed conversion observed in the MOS- and VM-fed turkeys during this study, and the results observed by other researchers. Buresh et al. (1985) demonstrated that VM significantly reduced the amount of energy required to produce a gram of weight gain, especially for poult's consuming a restricted energy diet compared to birds consuming an *ad libitum* diet. Harms et al. (1986) reported that VM improved dietary energy utilization far greater in low energy content diets compared to diets rich in energy. Dietary MOS may also improve dietary energy utilization, but likely by a different mechanism than antibiotic growth promoters.

Gut Brush Border Morphology. The effects of antibiotics and MOS on gut microflora, and improved nutrient utilization and growth performance may be associated brush border morphology and how it influences enteric disease resistance. To test this hypothesis, an experiment was designed to ascertain effects of MOS and VM on jejunum villi morphology. Commercial Hybrid<sup>®</sup> poult's were subjected to three dietary treatments starting a 1 day of age: 1) corn-soybean meal control diet; 2) MOS (1 kg/ton); and 3) VM (20 g/ton active ingredient). At 14 days of age, 8 birds per treatment pen were sampled for morphometric analysis of 5 cm sections of jejunum. Measurements of villus height, crypt depth, muscularis thickness, and goblet cell number were made at a magnification of 10X. A minimum of 15 measurements per slide were made for each parameter and averaged into one value per bird, which was then used for statistical analysis. The morphological observations of jejunal brush border are summarized in Table 4.

MOS had the greatest effect on villi morphology. Although MOS did not affect villus height, a decrease in crypt depth approached significance and villi height: crypt depth ratio was significantly greater than the control or VM treatments. Iji et al. (2001) also observed an increase in jejunal villi height: crypt depth ratio by MOS supplementation in broilers, but this was due to a significant increase in villi height rather than crypt depth. These researchers also observed MOS to significantly increase protein/DNA of jejunal mucosa, as well as increase in the brush border enzymes maltase, leucine aminopeptidase and alkaline phosphatase. Turkeys receiving 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. As observed with older toms detailed above, the poult's consuming VM had a significantly lower jejunal muscularis weight than the control treatment, while an increase in goblet cell count per mm villus height approached significance ( $P < 0.10$ ).

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 MOS resulted in an increased proliferation of goblet cells into the surface of the villus membrane. The innate immune system has developed to recognize 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 (Janeway, 1993; Newman, 1994). Intestinal microbes might influence goblet cell dynamics by releasing

bioactive compounds or indirect activation of the immune system (Bienenstock and Befus, 1980). Contrary to expectations, dietary VM increased goblet cell numbers in the poult. Antibiotic therapy was expected to decrease host reliance on mucus secretion for protection. However, decreasing numbers of viable Gram-positive bacteria, such as *Lactobacilli* and *Bifidobacteria*, may increase the presence of Gram-negative species, which may necessitate the need for more mucus production and hence more goblet cells (Edens et al., 1997).

Table 4. Effect of virginiamycin (VM) and mannanoligosaccharides (MOS) on the intestinal morphology of the jejunum of 14 d old hen poult<sup>1</sup>

Treatment	Villus Height	Crypt Depth		Muscularis Thickness	Goblet Cells	
		-----( $\mu\text{m}$ )-----			(No./villus)	(No./mm villus)
Control	905 <sup>a</sup>	104 <sup>a</sup>		163 <sup>a</sup>	104 <sup>a</sup>	116 <sup>b</sup>
MOS	823 <sup>a</sup>	86 <sup>a</sup>		128 <sup>b</sup>	137 <sup>a</sup>	169 <sup>a</sup>
VM	855 <sup>a</sup>	98 <sup>a</sup>		136 <sup>b</sup>	120 <sup>a</sup>	137 <sup>ab</sup>
P-value	0.30	0.08		0.01	0.07	0.01
SEM (19) <sup>2</sup>	36	6		8	10	10

<sup>a,b</sup> Means within a column with differing superscripts differ significantly ( $p < 0.05$ ).

<sup>1</sup> Means are an average of 15 individual measurements per bird and eight birds per treatment at 14 d of age.

<sup>2</sup> SEM(19) = Standard error of the mean with 19 degrees of freedom.

**Response to immunological stress.** All birds reared under commercial field conditions are subjected to immunological stress to some degree, depending on the pathogen load in their environment and the vaccination program. The positive effects observed among birds given antibiotic growth promoters or MOS may be partly due to how these feed additives influence a bird's response to acute immunological stress. In an experiment where birds were fed virginiamycin, MOS or control feed as described above, half of the birds were subjected to acute immune stress at 21 days of age and the other half was not. Acute immune stress was induced by intraperitoneal injection of 3 mL of sterile *Salmonella typhimurium* strain SL 684 LPS (100 mg LPS/L) in an 8.2 g NaCl/L solution. The control birds received saline injections. Cloacal temperatures were measured eight hours after LPS/saline injection, and then 24 hours post-injection body, liver, spleen, bursa of Fabricius, and intestinal tract weights were recorded.

The effects of *S. typhimurium* LPS challenge on fever response and lymphoid tissue are presented in Table 5. There were no dietary treatment effects observed among the non-injected birds (-LPS). LPS injection (+LPS) induced a mild fever response as indicated by a 0.25° C increase in body temperature. Challenge with LPS increased liver and spleen weights compared to non-injected birds, but it did not effect bursa and intestinal weights. Challenge with LPS increased liver (2.78 Vs. 2.44 g/100 g BW,  $P < 0.05$ ) and intestinal (3.67 Vs. 3.33 g/100 g BW,  $P < 0.05$ ) weights of birds fed VM.

Among the +LPS treated birds, significant dietary treatment effects were observed, indicating that diet can influence a bird's response to acute immunological stress. In contrast to the control and VM-fed birds, the MOS-fed birds showed no fever response 8 h post-injection, even though liver and intestine weights were increased. In other words, the MOS-fed birds retained normal body temperature after exposure to a pro-inflammatory antigen, while the controls and VM-fed birds expressed elevated body temperature. An increase in body temperature after antigen challenge is associated with IL-1 release (Klasing et al., 1987) and depressed growth. During immunological stress, feed intake decreases and nutrients are mobilized away from liver and muscle in favor of supplying the demands of the innate immune system's inflammation response. This pro-inflammatory response is far more "costly" to the bird in terms of nutrient availability for growth than a strong humoral immune response (increase in antibody titers) (Humphrey et al., 2002). Under commercial conditions where birds are subjected to chronic

immunological stress, MOS may help reduce the pro-inflammatory response and associated depression in feed intake and growth.

In previous studies, MOS have been shown to have a positive influence on humoral immunity and immunoglobulin status. Savage et al. (1996) reported an increase in plasma IgG and bile IgA in poult fed diets supplemented with 0.11% 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). Therefore, some MOS-immune system cross talk would be expected.

Table 5. Effects of mannanoligosaccharides (MOS), virginiamycin (VM), and MOS-VM shuttle program on live weight and rate of gain in female turkeys<sup>1</sup>

Immunological Stress	Dietary treatment			SEM(d.f.) <sup>2</sup>
	CON	MOS	VM	
	-----Cloacal Temperature (° C) 8 hr post-injection-----			
- LPS	41.40 <sup>a</sup>	41.49 <sup>a</sup>	41.38 <sup>a</sup>	0.11(19)
+ LPS	41.80 <sup>a</sup>	41.48 <sup>b</sup>	41.73 <sup>a</sup>	0.08(10)
	-----Liver Weight (g)-----			
- LPS	2.38 <sup>a</sup>	2.49 <sup>a</sup>	2.33 <sup>a</sup>	0.09(38)
+ LPS	2.44 <sup>b</sup>	2.78 <sup>a</sup>	2.45 <sup>b</sup>	0.10(38)
	-----Small Intestine Weight (g)-----			
- LPS	3.82 <sup>a</sup>	3.66 <sup>a</sup>	3.36 <sup>a</sup>	0.14(38)
+ LPS	3.33 <sup>b</sup>	3.67 <sup>a</sup>	3.14 <sup>b</sup>	0.11(38)
	-----Spleen Weight (g)-----			
- LPS	0.14 <sup>a</sup>	0.15 <sup>a</sup>	0.13 <sup>a</sup>	0.01(38)
+ LPS	0.16 <sup>a</sup>	0.17 <sup>a</sup>	0.15 <sup>a</sup>	0.01(38)
	-----Bursa Weight (g)-----			
- LPS	0.15 <sup>a</sup>	0.15 <sup>a</sup>	0.13 <sup>a</sup>	0.01(38)
+ LPS	0.13 <sup>a</sup>	0.13 <sup>a</sup>	0.14 <sup>a</sup>	0.01(38)

<sup>a-c</sup> Means with different superscripts within a row differ significantly ( $P < 0.05$ ).

<sup>1</sup> Values presented are means of 8 birds per dietary treatment.

<sup>2</sup> Standard error of the mean (degrees of freedom).

## FRUCTOOLIGOSACCHARIDES

Fructooligosaccharides are another class of oligosaccharide that can modify enteric microflora, but they function differently than MOS. FOS compounds are inulin-type oligosaccharides of *D*-fructose attached by  $\beta$ -(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 1<sup>F</sup>- $\beta$ -fructofuranosyl (Hidaka and Hirayama, 1991). Fructooligosaccharides are found in numerous plants such as the onion, Jerusalem artichoke, garlic, banana, chicory, asparagus, and wheat.

FOS influence enteric microflora by “feeding” the “good” bacteria, which competitively excludes the colonization of pathogens. FOS will not bind bacteria with type-I fimbriae as will MOS. Dietary supplementation of FOS provides selective enrichment of *Lactobacilli* (Mitsuoka et al., 1987) and

*Bifidobacteria* (Hidaka et al. 1986; Hidaka et al. 1991). Patterson et al. (1997) found that cecal *Bifidobacteria* concentrations were increased 24-fold ( $P < 0.001$ ) and *Lactobacilli* populations increased 7-fold ( $P < 0.007$ ) in young broilers fed the FOS-enriched diets. Fructooligosaccharides are well utilized by the majority of *Bifidobacteria* strains (*longum*, *brevis*, and *infantis*) with the exception of *Bifidobacterium bifidum* (Hidaka and Hirayama, 1991). The *Bacteroides* group also showed a tendency to utilize FOS as a growth source, while *Lactobacillus fermentum*, *E. coli*, and *Clostridium perfringens* failed to utilize FOS as a fermentative carbohydrate source. *Bifidobacteria* readily ferment FOS because of the innate secretion of a  $\beta$ -fructoside enzyme. *Bifidobacteria* may inhibit other microbes because of its acidic surroundings from the high production of VFA's or the secretion of bacteriocin-like peptides. Work presented by Hopkins et al. (1998) outlined the ability of *Bifidobacterium* species to flourish on fructooligosaccharides.

The majority of work that has been done in poultry has been reported on the feeding of FOS to broilers. 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. Ammerman et al. (1989) compared the use of FOS as a sub-therapeutic antibiotic replacement in male broilers reared from 1 to 47 days of age. The FOS treatment at 0.375% produced heavier birds at day 47 as compared to control-fed birds. Hot carcass weight and percent breast weight were also improved by the addition of 0.375% FOS, while percent fat pad was subsequently reduced in these birds. Patterson et al. (1997) conducted studies on the feeding of a FOS-type kestose oligosaccharide compound (structure containing a glucose molecule in conjunction with varying numbers of attached fructose molecules) to broilers. Birds fed a diet containing approximately 2% kestoses and 8% other sugars showed improved 28 day body weights (989 vs. 938 g) over controls and over birds fed a diet containing 8% of the other sugars (989 vs. 968 g), although these improvements were not statistically significant. There were no differences seen in other performance parameters. There are no published data on the effect of FOS on immunity and disease resistance.

## CONCLUSION

This paper reviewed the benefits and concerns about antibiotic growth promoters and the potential of oligosaccharides, such as MOS and FOS as non-pharmaceutical alternatives. Dietary antibiotics clearly promote efficient growth and health of poultry, but public concern about the increasing threats of antibiotic-resistant pathogens has forced the poultry industry to consider other compounds that do not result in the development of bacterial resistance. There is considerable evidence now that oligosaccharides, such as MOS and FOS may produce similar benefits as antibiotic growth promoters, especially if they are used strategically together with pharmaceutical or non-pharmaceutical enteric conditioners. Both antibiotics and oligosaccharides improve growth performance primarily by modifying the microflora ecosystem. Most antibiotic growth promoters are broad-spectrum inhibitors of gram-positive bacteria, which produce metabolites that are toxic to enteric tissues and cause immunological stress. In contrast, oligosaccharides increase the colonization of "good" bacteria (i.e. *lactobacilli spp.* and *bifidobacteria spp.*) that have a positive symbiotic relationship with the host. MOS has an additional advantage by inhibiting the colonization of pathogens while allowing the animal to express enhanced humoral immunity and enteric disease resistance.

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# NUTRITION FOR ALTERNATIVE POULTRY PRODUCTION

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## DEFINITIONS

### Organic

Organic foods are those grown using organic farming practices. Federal regulations have recently been introduced which define these practices. Under these new regulations, organic poultry or edible poultry products must be from poultry that have been raised under continuous organic production beginning no later than the second day of life. Organic feed must be supplied to the birds. The birds must have access to the outdoors, shade, fresh air and direct sunlight suitable to the climate and stage of production. Other terms used include: ecologically grown, or eco-foods, and natural (although no legal definition for 'natural').

### Free-range

In a free-range production system the birds are not confined but are free to range in a particular area. They are typically provided with evening shelter. It is not a clearly defined term and often simply refers to birds with 'access' to the outdoors. Free-range should be distinguished from cage-free or free-roaming, which are not synonymous with free-range.

### Pasture-raised

'Pasture poultry' is a term used for a modified free-range system. A floorless, field pen is used to hold the birds on pasture and the cages are moved on to fresh pasture daily. Feed and water are provided in the pen where the birds are also free to forage on plants, seeds, insects and worms. This is different than the 'chicken tractor,' which uses the birds to prepare the soil for garden plots rather than rotating them on pasture.

### Day-range

Day-ranging is a modified pasture-raised system. The birds are supplied with shelters that have floors with deep bedding. The system also uses portable electric poultry netting. This fencing material is used to steer the birds to the forage available for that day. The birds are locked within the shelter at night. With broilers, the temporary paddock typically remains in one location for the entire flock. The shelter and paddock are then moved for the next flock. For laying hens and turkeys, the paddocks are moved as needed.

### Label Rouge (Red label)

The Label Rouge system for raising broilers is popular in France. It uses lower bird densities, allows the flock access to the outdoors, discourages routine medication and features a slower growing broiler strain. It typically requires 12 weeks for the broilers to reach market weight.

### ORGANIC POULTRY PRODUCTION

The new federal standards for certified organic poultry and livestock production stipulated that the following ingredients NOT be included in the organic feeds:

- Animal drugs used to promote growth
- Feed supplements or additives in amounts above those needed for adequate nutrition and health maintenance for the species at its specific stage of life
- Mammalian or poultry slaughter by-products
- Synthetic parasiticides
- Products processed by ionizing radiation
- Genetically modified grain or grain by-product
- No chemically extracted feedstuffs such as solvent-extracted soybean meal
- No synthetic amino acids

Enzymes can only be used when they are derived from edible, nontoxic plants, nonpathogenic fungi, or nonpathogenic bacteria.

Organically-raised poultry must have access to the outdoors. The producer must show how they will maximize and encourage access to the outdoors. Birds raised on grass have the potential for impaction of the gastrointestinal tract leading to pendulous crop. This can occur despite the provision of grit.

### PASTURE POULTRY

Birds are typically placed on pasture at 3-weeks of age, but timing depends on the current weather. Birds raised on pasture have different nutritional needs than birds raised in conventional poultry houses. It is important to make sure that the feed contains enough fat to keep the birds warm.

The type of pasture used varies widely. The pasture can be any mixture of grasses, but many prefer legumes (clover/alfalfa) at heights of 4-8 inches. Higher heights make it difficult to move the pens. The birds also eat less. The more vegetative, the more grass they will eat. It is possible to graze cattle on the pasture a few weeks before used for poultry production. Such combinations have also been shown to be useful in controlling flies.

## ORGANIC POULTRY FEEDS

The availability of certified organic corn and soybean is expected to decline, or at least not keep up with demand. Solvent-extracted soybean meal cannot be used in certified organic poultry feeds. It is necessary, therefore, to formulate certified organic feeds without the use of corn and soybean meal, the two feedstuffs routinely used in commercial poultry feeds today.

Organic crops are grown without the use of synthetic fertilizers or pesticides (i.e., herbicides, insecticides, or fungicides). Instead, emphasis is placed on the use of organic soil amendments and the use of crop rotations that enhance the cropping system's natural defenses against disease, insects, and weeds. Crop rotation programs would encourage the production of crops other than the typical corn and soybean routinely used in commercial poultry feeds.

### Use of alternative crops in poultry feeds

#### Buckwheat

Buckwheat (*Fagopyrum sagittatum*) has long been used as a livestock and poultry feed, but, unfortunately, little data is available on its use. The literature suggests that buckwheat has reasonable feed value, roughly comparable to oats (Cheeke, 1991). The grain contains 11-13% crude protein and is the best source of lysine among the feed grains, and is the only one not lysine deficient. The proteins of buckwheat are of high biological value with essential amino acids making up over one third of the total protein (Pomeranz, 2000). Buckwheat grain is considered to have lower feed value than grain of cereal crops because of its relatively high fiber content and low digestible nutrients (Pomeranz, 2000). Buckwheat also contains fagopyrin, a compound which causes photosensitization of light-skinned animals.

#### Naked oats

Oat (*Avena sativa*) grains are composed of about 20% hulls, resulting in a high fiber/low energy grain. The hullless or naked oat, however, has a feeding value similar to that of corn (Cheeke, 1991). Naked oats (*Avena nuda* L.) contain about 17% crude protein with 0.68% lysine. The ME of naked oats is about 3200 kcal/kg, which is similar to that of wheat. Oat lipids have a high proportion of palmitic acid leading to a "harder" fat being deposited in the chicken carcass (Leeson and Summers, 1997). Oats (both regular and naked) contain beta-glucans, which can cause digestive problems and sticky litter when fed to poultry. Maurice et al. (1985) reported that up to 40% naked oats could be included in broiler diets with no adverse effect on growth, feed efficiency, shrinkage, dressing percentage or bone strength. Similarly, Cave and Burrows (1985) found that up to 60% naked oats could be included in broiler grower diets with no adverse effects on final body weights (compared to broilers fed corn-based diets). They did observe, however, a decrease in feed efficiency when naked oats were included at 60% of the diet. In contrast, Cave (1985) found that the inclusion of naked oats into broiler starter diets at levels greater than 20% resulted in decreased feed intake and weight gain. Hsun et al. (1986) speculated that the low weight gain of chicks on naked oat diets was due to the low availability of amino acids. In terms of product quality, Poste et al. (1996) found that at 50% inclusion in broiler diets, naked oats had

a negative effect on some sensory quality parameters (tenderness, juiciness and to some extent stringiness and rubberiness). This was not found at 25% inclusion. Hsun and Maurice (1992) reported that up to 66% naked oats could be included in layer diets with no adverse effects on egg yolk, feed intake, egg weight, or egg production.

### Barley

Barley (*Hordeum vulgare*) is commonly used in Canada and Europe as the major energy source in poultry diets, but is considered a low energy grain. The lower energy value of barley is due to a low starch content, a high fiber content, and the presence of beta-glucans. Beta-glucans cause low digestibility and sticky droppings. The sticky droppings create bad litter, which can cause hock problems and damage the breast of broilers. With layers, the sticky droppings adhere to the cage and mark eggs decreasing their quality and marketability. Barley is considered inferior to either corn or wheat as an ingredient in poultry diets (McNab and Smithard, 1992). Barley contains twice as many fatty acids as wheat, which accounts for its 10% higher caloric content. Barley contains over 17% fiber, which is 40% more than that in wheat. Birds fed diets based on barley have been shown to be more susceptible to necrotic enteritis than those on corn-based diets. The development of commercially available enzyme preparations have increased the use of barley in poultry feeds, but the use of feed enzymes is restricted in certified organic poultry production.

### Wheat

Wheat (*Triticum*) is also used in many countries as the major energy source in poultry diets, especially in Canada and Europe. Wheat varieties are classified as red or white depending on the seed coat color, hard or soft depending on the kernel texture, and winter or spring depending on time of planting. In terms of feeding value, the main classification of interest is kernel texture since this most strongly affects nutrient composition. Hard wheat varieties generally have a higher lysine content than soft varieties. The hardness of these wheats is due to the strong binding between the starch and the protein components. Within the hard wheats, protein levels can vary from 10-18% depending on the variety and growing conditions (Leeson and Summers, 1997). Wheat contains 5-8% pentosans (e.g., arabinoxylan), which can cause problems with digesta viscosity. Studies of the arabinoxylan in wheat have shown a reduction in nutrient digestibility and chick performance (Brenes et al. 1993). Wheat has a lower starch digestibility than corn (Maisonnier et al. 2001). As with barley, the development of commercially available enzyme preparations have increased the use of wheat in poultry feeds, but the use of enzymes is restricted in certified organic poultry production.

### Sorghum

Sorghum (*Sorghum bicolor*) is similar in composition to corn but contains the anti-nutritive factor tannin. Tannins (phenolics) inhibit digestive enzyme activity and form complexes with protein that resist digestion. The maximum amount of sorghum tannin that can be included in broiler diets without adversely affecting growth rate or feed efficiency is unclear. The results of Jacob et al. (1996) suggest that the maximum level is between 1.3 and 2.5% tannin on a dry matter basis. Sorghum is limiting in several amino acids, including lysine, methionine and

glycine (Gardiner, et al. 1981). There is very little data available on the effect of sorghum inclusion on meat yield and quality, but a study by Mohamedain et al (1986) showed a significant reduction in both eviscerated carcass weight and dressing out percentage of broilers reared on sorghum.

### Pearl millet

Millet is a collective term for seeds from a variety of crops including pearl millet (*Pennisetum glaucum*), foxtail millet (*Setaria italica*), and proso millet (*Panicum miliaceum*). Pearl millet has about 10-16% crude protein (Burton et al., 1972) and 3,300 kcal/kg of metabolizable energy (Adeola et al., 1994). Pearl millet is rich in oil with linolenic acid, which comprises 4% of the total fatty acid content. By comparison, corn is notably deficient in omega-3 fatty acids with linolenic acid comprising only about 0.9% of total fatty acids (Collins et al., 1997). Laying hens fed diets containing pearl millet were found to produce eggs with significantly higher omega-3 fatty acids (Collins et al., 1997). Adeola et al. (1994) reported that pearl millet could replace corn in broiler diets without any adverse effects on weight gain, feed efficiency, and feed conversion. One problem with millet, however, is finding a way to mill it. Many mills are set up to grind corn, not millet, which requires rolling, not grinding.

### Rye

Rye (*Secale cereale*) has a nutrient content comparable to that of wheat and corn but its feeding value is limited by the presence of anti-nutritional factors. Rye contains a higher pectin content than other grains and there is some evidence to suggest that the poor growth and sticky droppings of chicks fed rye are due to pectins. Pectins are a group of polysaccharides that are viscous and gummy in nature. Sub-therapeutic levels of antibiotics in the diet prevent the growth-depressing effects of rye in poultry, but such dietary treatments are not permitted in certified organic poultry production. Recently enzymes have been developed which will markedly reduce the anti-nutritional effects of rye. Enzyme supplementation decreases viscosity of intestinal contents and improves nutrient digestibility and absorption in broiler chickens fed diets containing rye (Friesen et al., 1991). However, as stated earlier, the use of feed enzymes is restricted in certified organic poultry production.

### Amaranth

Amaranth (*Amaranthus cruentus*) was a primary food for Central American Indians before Columbus arrived in the New World. The grain has a protein content of 14-18%. It is high in lysine and well balanced in other amino acids (Baltensperger et al., 1995). Raw grain amaranth contains heat labile, growth depressing anti-nutrients for chickens, although Japanese quail are not affected (Vohra et al., 1989). Grain amaranth can be used as a feed ingredient for broilers if heat treatment is applied to the grain prior to feeding (Tillman and Waldroup, 1986; 1988). The heat treatment is necessary to partially or completely destroy the anti-nutritive factors present. Tillman and Waldroup (1988) found that extruded grain amaranth can be fed to broiler chicks without adversely affecting body weight, feed utilization, or carcass yields. They also suggested that the upper limit of inclusion is less than 40%. Amaranth grain also has a market in the health

food industry where it is an alternative for those with allergies to wheat. If it is to be used in organic poultry feeds it will have to compete with this market.

### Flax

Flax seed contains high levels of protein (26%) and oil (41%). It is also an excellent source of omega-3 fatty acids, particularly linolenic acid. Flax is currently used in poultry feeds to alter the fatty acid composition of eggs (i.e., omega-3 enriched eggs). Leeson et al. (1998), however, found that inclusion of high levels of flaxseed (>10%) resulted in a decrease in overall egg acceptability as assessed by aroma and flavor. Current practice in feed formulation is to stabilize flaxseed with the addition of a tocopherol/vitamin E antioxidant at the level of 10 mg/100g of feed. The flavor quality of vitamin E/omega-3 fatty acids enriched eggs has been found to be superior to eggs solely containing enhanced omega-3 fatty acids. Flax seed has also been shown to be successful in the production of omega-3 enriched chicken meat, although the use of full-fat flax seed resulted in lower live weights and smaller carcasses (Ajuyah et al., 1991). Flax oil may also be an effective method of reducing ascites and pulmonary hypertension in broilers without affecting performance (Bond et al., 1996). Allen et al. (1997) studied the potential of flax seed to control coccidiosis. They demonstrated that feeding flax-supplemented diets to broilers suppressed *E. tenella* development, but was not beneficial in reducing the effects of *E. maxima* infections and may actually exacerbate lesions at high parasite doses. For flax seed to do any good, the seed must be broken open. The outer shell on the flax seed is so hard that unbroken, it just passes right through, retaining all its nutrients.

### Field peas

Field peas (*Pisum sativum*) contain 20-29% crude protein and are a potential protein-energy source for poultry diets (Cheeke, 1991). Guillaume (1977) reported replacement of a large portion of the soybean meal with field peas resulted in slightly reduced performance of growing chickens and laying hens. The presence of alpha-galactosides was proposed as the cause of the poorer growth of the chickens, but the cause of the reduced performance of the laying hens was not known. Igbasan and Guenter (1996) reported that broiler chickens could tolerate up to 20% field peas in their diets. Up to 40% field peas could be used if the diets were supplemented with the enzyme pectinase and essential amino acids up to 15% in excess of NRC requirements. Similarly, Farrell et al. (1999) recommended maximum inclusion rates of 30%. Peas, as with other legumes, are low in methionine. In addition, some varieties have high levels of tannin. Igbasan et al. (1997) reported high levels of starch in peas, however, pea starch is less digestible than the starch of any cereal grain.

### Sunflower

Sunflower (*Helianthus annuus*) seeds are used for oil production. The meal remaining after oil extraction is a potential feed ingredient for poultry. Sunflower meal has a relatively high protein content (17-21%) (San Juan and Villamide, 2001) but is low in energy and deficient in lysine, limiting its use. Solvent extracted sunflower seed meal cannot be used in certified organic poultry feeds. It is also possible to include whole sunflower seeds in poultry diets. Uwayjan et al. (1983) found that unprocessed whole sunflower could be included at up to 30% of layer diets

with no adverse effects on hen performance. They did observe, however, that diets containing sunflower seed gave a significantly lower yolk color score and a significant rise in yolk cholesterol content. Cheva-Isarakul and Tangtaweewipat (1991) reported adequate weight gain and feed efficiency when whole sunflower seeds were incorporated at up to 50% of broiler diet. The seeds were ground for inclusion in the diets.

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# FACTORS THAT AFFECT FEED INTAKE OF MEAT BIRDS

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## INTRODUCTION

The amount of feed intake is very closely associated with growth performance in meat-type poultry. Modern commercial broilers and turkeys will not grow to their full genetic potential unless they consume their full nutritional requirement each and every day. Aside from adequate diet formulation, maintaining maximum feed intake is the single-most important factor that will determine the rate of growth and efficiency of nutrient utilization. Flocks that exhibit the top average daily gain almost always have the highest feed intake and often have the best feed conversion and livability rates. Therefore, successful poultry integrators and growers must be keenly aware of the many factors that influence feed intake.

Feed intake control is a complicated interaction of many factors that involve a bird's physiology, sensory systems, and nutritional needs to meet the demands of growth, maintenance, and disease resistance. Feed intake is influenced by both dietary and management factors. Dietary factors can be subdivided into matters of dietary nutrient composition, feed formulation and feedstuff inclusion levels, and feed pellet quality. Management factors that influence feed intake can be subdivided into matters of feed and water availability to the birds, environmental management, stocking density, and disease control. This presentation will briefly discuss how each of these dietary and management factors influence feed intake.

## FEED INTAKE CONTROL

The fundamental physiological theories of feed intake control and appetite regulation have been studied primarily in mammals yet very little information exists on poultry (Gleaves, 1989). The control of feed intake in mammals is attributed to the hypothalamic region of the brain, which responds to various sensory stimuli and regulatory mechanisms. In contrast to mammals, visual and textural properties of food has a much greater influence on feed intake of birds than taste or smell. The bird will not readily consume feed if it does not recognize it as food by visual means. Of course food recognition is dependent upon previous exploratory experiences. The following will briefly discuss the sensory and physiological regulatory factors that influence appetite or feed intake.

## Sensory factors that influence feed intake

The sensory aspects of feed intake can be categorized in three basic stages of food ingestion: 1) food recognition; 2) food prehension and ingestion; and 3) gastrointestinal activity.

Food recognition in poultry primarily involves vision. Newly-hatched birds have an innate preference for food of certain colors. Hess (1956) reported a bimodal color preference with peaks in the orange and blue region of the visual spectrum. A preference for green over red was found in chicks (Capretta, 1969) and turkeys (Cooper, 1971), which is the reason Oasis® (Novus International, St. Louis, MO) used to stimulate feed intake initiation of hatchlings is colored green. Young poultry have a natural curiosity to explore green-colored material as a potential source of food. Poultry also have an innate preference for food with certain shape and size (Gentle, 1985) similar to small seeds. However, poultry also have a strong bias to use vision (shape and color) in learning situations. For example, birds will learn to avoid a substance based on vision characteristics if it produced an illness or other unpleasant post-ingestion effects (Gillette et al., 1980). This illness-induced feed aversion is particularly detrimental in commercial meat bird production if they are given a bad batch of feed that produced ill effects. In contrast, an increase in food intake is associated with positive visual-tactile-gustatory stimuli from the food and the positive long-term effects of the food ingestion. It is important to associate good textural characteristics of feed (good crumbles and pellets) with good nutrient composition and diet formulation. Finally, social facilitation is an important aspect of feed intake among poultry. Chicks will initiate more pecking behavior when they see other birds exhibiting this behavior. Feed intake in a group of birds is often synchronized at specific times during the day even though feed is available *ad libitum* (Huges, 1971).

Chickens and turkeys are seed-eaters and the efficiency of feed intake is greatly dependent upon the particle size and shape that complements the physical attributes of the bird's mouth. They have difficulty consuming food that is too large or too small relative to the dimensions of its beak. Chicks and turkeys do not have teeth, so large particles cannot be "bitten" and divided into smaller ones. Although fowl canprehend fine feed, they cannot do it efficiently without significant feed wastage. Moreover, they must work more to consume a fine feed than pelleted feed, essentially reducing the productive energy of the feed. Because of the high degree of keratinization of the beak, birds have very little ability for oral manipulation of food. Feed particles must first be picked up and positioned by the beak and then a forward thrust of the head along with particle release moves the food to the back of the mouth where it is coated with a viscous saliva before swallowing. If the feed is too finely ground and not properly pelleted, it interacts with the saliva and forms a sticky mass that solidifies and interferes with prehension, especially when the diet contains wheat or other small grains. Feed prehension and intake will also be compromised by any alteration to the integrity of the beak, such as excessive debeak trimming used to manage flock cannibalism, or lesions due to fusariotoxin T-2.

Feed consumption in the bird is perceived by the bird *via* mechanoreceptors, thermoreceptors, and chemoreceptors in the mouth. Mechanoreceptors help the birds rapidly discern a feed's quality by its textural properties. Thermoreceptors in chickens respond to cooling of the surface of the beak and oral epithelium, but not to warm food temperature (Gentle, 1985). The chemoreceptors in fowl are clustered into taste buds. Chickens have an average of about 360 taste buds (Saito, 1966), 54% located in the palate, 42% in the floor of the mouth, and only 4% in the tongue. This taste bud distribution in the mouth is directly associated with the contact time of the food on the different areas of the mouth to enable better gustatory discrimination (Berkhoudt, 1977). Even though fowl have far less number of taste buds than mammals, they do have an acute sense of taste and changes in taste (Gentle, 1975). Kare and Maller (1967) demonstrated that chickens fed low energy diets have a marked preference for sucrose solution. Hughes and Wood-Gush (1971) found that chickens would quickly select calcium carbonate-supplemented diets when they are calcium deficient. Similar responses to other nutrient deficiencies are likely. In condition-aversion studies, birds are more responsive to weakly-flavored foods than strongly-flavored foods (Gillette et al., 1983), indicating that a bird's sense of taste can be overwhelmed.

#### Theories of feed intake regulation

Once feed is consumed, there are several possible mechanisms that regulate feed intake. The common regulatory mechanisms of feed intake include: the glucostatic theory, the thermostatic theory, distention of the gastrointestinal tract, circulating amino acids and protein intake, the lipostatic mechanisms (Gleaves, 1989). The glucostatic theory ascribes to the regulation of blood sugar and the amount of glucose entering the liver after a meal. Shurlock and Forbes (1981) observed reductions in feed intake after they infused glucose into the hepatic portal vein of fasted chickens at physiological rates, whereas no effect was observed when glucose was infused into the jugular vein. The glucostatic control mechanisms seem to have a priority over all others as birds tend to consume feed to satisfy their energy requirement first. The second priority is to consume feed to satisfy daily amino acid intake requirements. Under free-choice feeding conditions where different dietary sources are available, birds will modulate their feed intake to satisfy both energy and daily amino acid needs. In commercial conditions where there is only one choice of feed available, feed intake is greatly influenced by both the dietary energy and amino acid profile.

The thermostatic theory is closely associated with a bird's thermal regulation. Feed intake decreases as ambient temperature rises above thermal neutrality (Hurwitz et al., 1980). Because the metabolic processes associated with digestion add significantly to the body heat load, feed intake must decrease to maintain body temperature when the birds are exposed to chronic heat stress conditions. The latent heat of digestion is dependent upon diet composition. Dietary energy in the form of carbohydrate generates significantly more latent heat of digestion because of active transport than when energy is in the form of dietary fat. Likewise, providing the bird with an ideal amino acid balance will generate less metabolic heat than a poorly balanced diet because less excess amino acids must be catabolized. Therefore, feed intake of birds reared under heat stress

conditions can be optimized by increasing dietary fat at the expense of carbohydrate and protein, and using supplemental amino acids to improve dietary amino acid balance. The thermostatic control of feed intake may also be important in birds experiencing a fever response during an innate immune challenge. Pro-inflammatory cytokines cause an increase in basal metabolic rate and body temperature as nutrients are mobilized away from growth to support the immune system (Koutsos and Klasing, 2001). Consequently, feed intake decreases to alleviate any additional metabolic heat load during the fever response similar to the decrease in feed intake during a heat stress condition.

Gut distension and gut motility most likely influence feed intake of birds, but relatively little is known about how gastrointestinal activity influences feed intake in comparison to other factors. Hodgkiss (1981) demonstrated the presence of two types of distension-sensitive receptors in the crop: slowly-adapting receptors and rapidly-adapting receptors. The slowly-adapting receptors signal distension of the crop for prolonged periods of time and are associated with feed transit rate. The fast-adapting receptors are associated with meal feeding behavior. Distension receptors also exist in the gizzard (Duke et al., 1977) and helps control food grinding and gut motility. Distension receptors in the duodenum are associated with sensing osmotic pressure.

Gut distention and tactile receptors in the gizzard, play an especially important role in the control of gut motility. The gizzard is the “pace-maker” of normal gut motility (Duke, 1994). Unlike mammals, vigorous gut reflexes (reverse peristalsis) are normal in birds as an adaptation to compensate for a short intestine. The reflexes serve to re-expose intestinal digesta to gastric secretions, vigorously mix digesta with enzymes to enhance digestion, enhance nutrient absorption over a short segment of the gut, and discourage microbial proliferation that may cause disease or compete for nutrients. Poor gut motility will result in an unstable gut microbial ecosystem, increase the severity of enteric disease, and thus cause significant reductions in feed intake (Ferket, 2000).

The lipostatic theory of feed intake control likely plays an insignificant role in juvenile meat poultry in comparison to older birds. As in many other species, adolescent and adult birds defend changes in body fat content by modulating dietary energy intake or energy expenditure. In other words, birds have a certain body fatness set point and their intake of energy will increase to the point where this minimum body fat content is reached. Because modern meat birds have been selected for weight gain and feed conversion with minimal control on body fat content, this body fatness set point has apparently drifted upward as evident by the observed increase in body fat. According to the lipostatic theory of feed intake control, the modern broiler has become hyperphagic in order to accommodate the genetic propensity for high body fat content. This is one reason why the feed intake of broiler breeders must be restricted to keep body fat at acceptable levels to support optimum reproductive performance.

#### DIETARY FACTORS THAT INFLUENCE FEED INTAKE

There are several dietary factors that influence feed intake, especially if dietary nutrient composition is either deficient or in great excess relative to the bird's requirement.

Because meat-type poultry have been selected for body weight gain, they are less responsive to dietary influences on feed intake than laying hens. Meat-birds tend to consume to maximum gut fill if they are not limited by dietary toxicities, environmental, management, or disease factors. The following discussion will address the major dietary factors that may influence feed intake.

### Dietary Energy

Dietary energy content has the most predictable effect on feed intake on meat birds. As mentioned in my discussion about the lipostatic theory of feed intake control, birds will attempt to consume feed to meet their metabolic energy requirement. Energy requirement is dependent on the energy needs for body maintenance and growth or production. Body maintenance requirements, which have a priority over productive needs, are influenced by the bird's health status, its degree of mobility (influenced by stocking density, physical activity, and social interactions), and body heat loss (influenced by ambient temperature, humidity, local air speed). Therefore, feed intake will increase as dietary energy content decreases until it is limited by either gut fill or other physiological limits. Because feed conversion is economically important in the raising of meat-type poultry, it is unpractical to stimulate increased feed intake by reducing caloric density. Limitations in feed intake are almost always associated with factors other than dietary energy content.

### Dietary Protein and Amino Acids

Dietary protein and amino acid content has more of an indirect effect on feed intake than any direct effect. Body weight gain will decrease as dietary amino acid content decreases below the level of requirement for optimum growth. As body weight decreases, the bird's caloric requirement decreases and consequently feed intake to meet this energy need decreases. Dietary amino acid imbalances due to poor feed formulation or poor feed ingredient digestibility will also cause decreases in feed intake and losses in feed conversion efficiency. Unlike the effect of dietary energy, meat birds will not modulate their feed intake to satisfy their amino acid requirements unless there is a slight deficiency in the first limiting amino acid. In such cases, increases in feed intake will be associated with a decrease in feed conversion efficiency.

### Dietary Vitamins and Minerals

Vitamins and minerals function primarily as cofactors of metabolism, while macrominerals, such as calcium, phosphorus, and magnesium also serve as structural components of the body. Vitamins and minerals influence feed intake only when dietary levels are deficient or several fold above requirement. Deficient dietary levels cause metabolic disorders that cause an indirect adverse effect on feed intake. Slight mineral deficiencies may stimulate feed intake as the bird attempts to achieve its intake requirement. In contrast, excessive dietary vitamins and minerals are detected by the bird's sense of taste, resulting a refusal to consume the feed. Mineral excesses are also associated with significant increases in water consumption. Excess in dietary salt will

depress feed intake and stimulate water consumption. Excess in dietary calcium will also depress feed intake in growing meat poultry.

### Antinutritional Factors

Nature has endowed many members of the plant kingdom with the capacity to synthesize unique chemical substances that serve as nutrient storage compounds or defense mechanisms. Many of these compounds can result in detrimental or fatal consequences if toxic quantities are ingested by poultry. Naturally occurring compounds such as protease inhibitors, goitrogens, alkaloids, oxalates, and phytates are innate natural components of particular feed ingredients that can impair the availability of nutrients, depress feed intake, and reduce the growth in animals that consume them (Hathcock and Rader, 1994; Shahidi, 1997). Other antinutritional factors in foods are produced as a result of fungal or microbial metabolism or by the plants themselves as defensive mechanisms against injury or infection. Fortunately, the presence of a toxic factor per se does not preclude the utilization of the material as a feedstuff. Numerous processing methods are available to neutralize or detoxify the deleterious components of by-products and waste materials. In addition, no toxic material is detrimental at any level of consumption. Provided the level of consumption is low, even the most toxic compounds can be tolerated without any adverse consequences (Yannai, 1980). A more detailed review of the effects of antinutrients on feed intake and growth performance in poultry was published by Ferket and Middleton (1999).

### Water Consumption

Water is the most essential nutrient of the bird's diet, although a requirement value cannot be easily determined as with other nutrients. The water requirement of meat birds depends on the environmental temperature and relative humidity, the composition of the diet, growth rate, and the efficiency of water resorption by the kidney. Water functions in the body as a solvent in which nutrients are transported about the body and waste products are excreted. Many of the chemical reactions brought about by enzymes take place in solution and involve hydrolysis. Because of the high specific heat of water, large changes in heat production can take place within the bird with very little alteration in body temperature. Water also has a high latent heat of evaporation, and its evaporation from the bird is a crucial part of thermal regulation. Considering these important functions, it is evident that adequate water intake and body hydration is an important influence on feed intake.

Meat birds drink at least twice as much water as the amount of feed consumed on a weight basis. Actual water consumption relative to feed intake varies depending environmental temperature and dietary factors. Increasing dietary crude protein increases water intake and water:feed ratios (Marks and Pesti, 1984). Crumbled or pelleted feeds increase both water and feed intake relative to mash diets, but water:feed ratios stay relatively the same (Marks and Pesti, 1984). Increasing dietary salt and other osmotically active minerals increases water intake (Marks 1987) in attempt to flush excess minerals via the kidneys.

Water consumption has its most profound effects on feed intake only when water consumption is restricted to the point that it begins to affect body hydration. Water availability is dependent upon stocking density and access to drinker space, drinker placement and height, drinker design, and water flow capacity. Although nipple drinkers are efficient and sanitary, they do not provide sufficient water flow for turkeys beyond 6 weeks of age, and consequently feed intake rate is adversely affected.

## MANAGEMENT FACTORS THAT INFLUENCE FEED INTAKE

Feed intake can vary significantly among flocks or different housing facilities even if they are all consuming the same feed and follow similar general management practices. These differences are almost always associated with differences in management and disease challenge. There are three general management factors that can have additive effects on feed intake of meat birds: 1) access to feed and water; 2) environmental stress; and 3) disease challenge. The relative effect of each of these factors on feed intake is dependent upon the stocking density. As stocking density increases, there is an increase in demand and competition for the basic resources: feed, water, litter, and air. Attention to each of these general factors is critical for the birds to consume the amount of feed at their genetic potential. The following is a brief discussion of each of the management factors that have the greatest impact on feed intake.

### Access to feed and water

Meat birds must have free, unimpeded access to feed and water whenever they want it from the day of placement until the day they are sent to market. Sufficient feeder and drinker space must be provided so that there is minimal competition among feeding birds. The feeders and drinkers must never be fully occupied throughout the day because the submissive birds within the flock will not be able to consume their required feed intake *ad libitum*. A high variability in flock body weight is an indication that there is not enough feeder space per bird. Even if there is sufficient feeder space per bird, placement of the feeder lines must be such that all the birds can easily access the feed without excessive maneuvering through a crowd of other birds.

Feeder and drinker height must be adjusted properly to allow each bird to easily access the feed without excessive spillage. Feeders that are adjusted too high will discourage the smaller birds from optimum feed intake, resulting in further degradation of flock uniformity. Make sure the litter level under the feeder lines does not have excessive hills and hollows which in effect increases variability in feeder height.

Proper feeder design and adjustment of feed flow for each pan is important for birds to easily access feed when they eat. The feeder design must suit the size of the bird, with proper grill wire spacing, feed flow and depth in each pan, and timely refilling. Too often, several feeders within the feed line are empty because of poorly adjusted feed flow, or the feed line operation switch in the last feeder is not functioning properly.

Finally, meat birds must never be without feed because these birds will not be able to compensate for lack of feed by increasing their feed intake at a later time. Each bird consumes feed according to a routine feeding schedule throughout the day and feed outages will disrupt subsequent feeding schedules and increase flock competition. Feed outages that extend beyond 4 hours will increase susceptibility to enteric disease that greatly compromise appetite and feed intake. Young birds are especially susceptible to feed outages.

### Environmental Stress

Stress has adverse effects on feed intake of meat birds. Elevation of the stress hormones associated with the stress response causes body reserves to be mobilized to fuel the "fight or flight" response. Nutrient absorption and gut motility decreases substantially during the stress response and feed intake decreases accordingly. Although acute stress may cause a momentary decrease in feed intake with minimal impact on performance, chronic stress will have a marked and persistently detrimental effect on feed intake. In general, chronic stress can be influenced by three environmental stressors: heat stress; poor air quality; and poor litter quality.

Heat stress clearly has adverse effects on feed intake of meat birds. The degree of heat stress endured by a bird is dependent upon several factors, including the body size and growth rate of the bird, ambient temperature and relative humidity, and the amount of convective heat loss as influenced by air speed. According to the thermostatic theory of feed intake control, birds will decrease their feed intake to reduce the heat load of digestion. Indeed, feed intake restriction prior to a period of high environmental temperature is an effective method of preventing excessive heat stress mortality, but this is not the most-productive method during chronic hot weather. Maintaining optimum feed intake and body weight gains during hot weather requires management techniques that promote heat dissipation by the birds. This may include stimulation of water consumption, increased airflow around the birds, and frequent misting in the house.

Feed intake will increase as ambient temperature decreases below the bird's thermal comfort zone, which depends upon its feather cover, body size, and daily rate of weight gain. For example, the minimum ambient temperature of comfort for a 3-week old poult is about 80 F, whereas it is about 55 F for 12- to 16-week old toms expressing their maximum rate of body weight gain. Below these minimum temperatures of comfort, the increase in feed intake becomes associated with losses in feed conversion.

Poor air and litter quality are environmental stresses that will indirectly depress feed intake. Ventilation rate and the litter management are the predominant determinants of air and litter quality. Adequate ventilation reduces air moisture, dust, ammonia, and carbon dioxide and brings in more oxygen. High air moisture decreases evaporative cooling and thus adversely affects feed intake in response to an increase in sensitive heat load. Excess air dust causes inflammation of the pulmonary system and immunological stress, which depresses feed intake as explained further below. Excess ammonia not only irritates pulmonary tissues, but it also is a metabolic stressor that causes depressed feed

intake. Finally, high levels of carbon dioxide or low oxygen levels in the air results in depressed metabolic rate that ultimately causes depressed feed intake. In addition to adversely affecting air quality (increases ammonia volatilization and dust), poor litter quality is also a medium of many pathogens that challenge the health status of the flock.

### Disease Challenge and Immunological Stress

Immunological stress caused by a disease challenge has a rather profound and significant effect on feed intake. Although enteric disease has obvious effects on reducing feed intake, any antigen (pathogen or vaccination) that mounts an immune response will depress appetite. The innate immune response is more nutritionally demanding and adverse to feed intake than the acquired immune response. The pro-inflammatory cytokine cascade associated with the innate immune response directly modulates the bird's behavior. Lethargy, reduced social interactions, and anorexia result from the actions of IL-1 and TNF- $\alpha$  on the brain and basal metabolic rate and fever. These behavioral changes results in reduced feed intake and body weight gain (Koutsos and Klasing, 2001). In addition to reduced feed intake, the nutritional status of a bird mounting an immune response is compromised by reduced absorption of specific nutrients. For example, water, sodium, chloride, and glucose absorption is reduced significantly by sepsis and is often associated with diarrhea (Kanno et al, 1996). About 70% of the reduced performance that occurs during an infectious challenge can be attributed to decreased feed intake and the remaining 30% is due to inefficiencies of nutrient absorption and utilization (Klasing et al., 1987).

Immunological stress has a marked effect on the hormonal milieu of poultry. The pro-inflammatory cytokines decrease anabolic hormones, such as growth hormone (Elsasser et al., 1997), insulin-like growth factor 1 (IGF-1) (Elsasser et al., 1995), and increases the release of catabolic hormones, such as glucocorticoids (Elsasser, 2000). The catabolism of skeletal muscles is further exacerbated by the reduction of IGF-1 associated with the decreased feed intake. However, once the bird has mounted an effective immune response and the pathogen is cleared, the pro-inflammatory cytokines declines and feed intake increases to normal levels or higher, ensuing a period of compensatory growth. In situations of chronic immunological stress, feed intake never has a chance to return to normal levels.

### CONCLUSION

Feed intake is the major factor that influences both the body weight gain and feed conversion in meat-type poultry. Because so many factors can influence feed intake, it is often difficult to correct problem of poor feed intake unless a complete review of feed and management practices is made. Management and flock health issues are usually more likely to cause feed intake problems than dietary factors. Dietary factors that influence feed intake would be common among all flocks in a complex rather than on individual flocks. In contrast, environmental or immunological stresses have the most profound effects on flock-to-flock variation of feed intake. Any management protocol that would alleviate these stressors will improve feed intake. For greatest success in

improving flock feed intake, start with investigating the sources of greatest stress or disease challenge.

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# EXOGENOUS ENZYMES FOR PIG AND POULTRY FEED RAW MATERIALS

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## 1. INTRODUCTION

The use of exogenous enzymes in pig and poultry diets is widespread around the World. There are perhaps two main reasons for this:

- (a) to improve the nutritional value of diets and raw materials (which, by definition, will also lower excretory losses and therefore have beneficial environmental consequences both for the animal and bird, and the environment itself through lowering pollution loads)
- (b) to reduce the variability in nutritional value between samples of the same named raw material (commercial nutritionists do not appreciate having to change raw material matrices).

However the mechanisms by which exogenous enzymes work are more diverse than simply hydrolyzing a substrate to its component monomers. In fact, this may not be particularly beneficial as some monomers (for example xylose) are of little metabolic value. Partial hydrolysis of a cell wall allowing the release of constituent substrates is another mode of action as is partial hydrolysis of water soluble non-digestible components thus reducing viscosity of digesta (leading to more efficient general enzyme action / nutrient uptake and altering microflora) which is being considered increasingly as the main benefit of exogenous enzyme use; it is the latter which is becoming accepted as the major benefit of use of exogenous enzymes.

It would be quite impossible to present a comprehensive review of published papers mainly because there are so many of them but also because a large proportion of individual trials are poorly conducted and data generated unique to the circumstances of the trial; enzyme effects are often difficult to reproduce because of variability in trial design and conduct. Accordingly, this paper will address some of the key issues in enzyme use in terms of the principles. Phytases will not be considered as they are the subject of a parallel symposium.

The fundamental principle behind exogenous enzyme use is that the diet / raw material contains a component which is not digested by the animal or bird which lack the specific endogenous enzyme necessary. There does need to be a qualification to this statement as, particularly in poultry, it is the speed of endogenous enzyme hydrolysis which is important. Thus endogenous  $\alpha$ -amylase may be present but not in sufficient quantities.

In the case of dietary enzyme addition, species and age of animal, diet formulation, site of enzyme action in the digestive tract and feed passage time will all affect the degree of hydrolysis possible. Enzymes in animal feeds can be derived from microbial, plant and animal sources but the majority are from bacterial and fungal fermentation. Individual fermentation cultures are known to produce a complex mixture of enzyme activities which are characterized by a multiplicity of enzyme forms and substrate specificity.

Enzyme sources are selected on the basis of substrate and reaction conditions. Since many substrates are complex, feed enzymes are usually cocktails and often contain activities which may not have been characterized accurately. Feed enzymes are marketed in dry and liquid forms and are applied during feed mixing or after pelleting. The final decision on form and method of application must consider enzyme stability, accurate application and even distribution in feed. Enzyme additives destined for animal feeds are crude preparations and generally are active over a range of substrates. Since most feeds are not usually based on a single cereal and legume source, this is not necessarily undesirable commercially although is a problem scientifically.

## 2. CHEMICAL COMPOSITION – SUBSTRATES OF NUTRITIONAL IMPORTANCE

### A. Cereals

Non starch carbohydrates (usually referred to as non starch polysaccharides – NSPs) are based upon ten different monosaccharide residues which are building blocks of cell walls of higher plants (Aman & Graham 1990). The ten are arabinose and xylose (pentoses); glucose, galactose and mannose (hexoses); rhamnose and fucose (6-deoxyhexoses) and galacturonic, glucuronic and 4-O-Me-glucuronic acid (hexuronic acids). The chemical features of NSPs are highly variable, depending on many factors including molecular weight, nature of monomers and types of linkages. NSPs can be classified as either water-soluble or insoluble. The former are nutritionally relevant whereas the latter, although predominating, are more inert in this context. However, it should be mentioned that, because of their hydrophilic nature, NSPs of quite large molecular size can dissolve in water; the molecular weight of water-soluble NSPs is rarely lower than 2000.

Separation of NSP into soluble and insoluble components will however depend very much on the analytical procedures adopted (Graham et al .1988). Furthermore, because these complexes are continuously modified during their passage through the gastro-intestinal tract, it is impossible to simulate this process with a simple chemical analysis of the feed (Aman and Graham 1990).

The physiological effects of plant cell wall polysaccharides have placed more emphasis on the definition and analysis of "fibre". Originally dietary fibre was defined as the remnants of plant cell walls which are resistant to digestive enzymes. This definition was later expanded to include indigestible NSP and lignin. However, the definition does exclude indigestible cell wall protein, cutin and waxes. Resistant starch and unavailable oligosaccharides are also considered by some as dietary fibre and hence there is still controversy as to the definition of this fraction (Aman and Graham 1990).

A major problem in research on plant cell wall polysaccharides has been the evaluation of suitable analytical methods. The determination of the molecular size of polysaccharides involves measurements of properties such as osmotic pressure, behaviour on ultracentrifuging and viscosity and gel permeation chromatography (Bedford & Classen 1992). Procedures for the analysis and chemical characterization of water soluble and insoluble dietary fibres (including NSP and Klason lignin) and hence total dietary fibre have been thoroughly researched and a wide variation in chemical composition has been found depending on the feedstuff in question (Table 1; Theander, et al. 1989). Even so, the yield and composition of soluble fibre can vary considerably with the extraction conditions and sample, hence the use of standardized and physiologically more appropriate extraction conditions was proposed by Graham, et al. (1988).

Table 1. Variation in content and composition of cereal 'dietary fibre' (DF)

Component g/kg DM		Mean Value	Range Values
Oats (n=16)	Total DF	296	198 – 387
	$\beta$ -glucan	32	27 – 36
	Arabinoxylan	80	41 – 145
	Cellulose	91	61 – 129
Barley (n=16)	Total DF	190	159 – 248
	$\beta$ -glucan	34	24 – 42
	Arabinoxylan	70	55 – 113
	Cellulose	53	30 – 73
Wheat (n=24)	Total DF	111	100 – 138
	$\beta$ -glucan	8	7 – 10
	Arabinoxylan	60	53 – 68
	Cellulose	25	18 – 34

Theander et al (1989)

The major NSP's in cereal grains are: (a)  $\beta$ -glucans which are linear polymers of sections of three or four  $\beta$ -1,4-linked glucose molecules, interspersed by two  $\beta$ -1,3-linked glucose components which make the polymer less tightly folded and hence

partially soluble in water. This solubility appears to be determined by the exact sequence of linkages of the polymer and its associations with other insoluble cell wall components; (b) Arabinoxylans which are arranged as a linear (1,4)- $\beta$ -xylan backbone with arabinofuranosyl residues coupled to the C2 and/or C3 hydroxyl groups; (c) Cellulose is a linear chain of  $\beta$ -(1-4)-linked glucose units, producing strong inter- and intra-molecular hydrogen bonding, which render this carbohydrate insoluble and resistant to enzymatic hydrolysis; it is therefore inert nutritionally.

There is considerable variation between different cereal types in content of NSPs (see Table 1) and also within types based on both genetic and environmental factors. In summary, NSPs in cereals are a complex group of components, often associated to each other or to other fractions, with different properties and physiological activities. Although plant cell walls are composed of a number of NSPs, these are not present in isolation of each other. Thus effective hydrolysis of the plant cell wall would require the simultaneous presence of many enzymes. A further outcome of this complexity is that studies using isolated and purified NSPs to demonstrate nutritional and physiological effects may not be valid as such procedures assume that the isolate behaves in the same manner as when a component of the overall plant cell wall complex; such an assumption is in all likelihood not probable.

## B. Legumes

As a result of the animal protein ban throughout the European Union, attention is focussing increasingly on the use of legume proteins to supply amino acids to poultry and pigs. Although there is a modest level of production of legumes grown in the EU (peas, lupins) by far the most important crop is the soya bean which is imported from North and South America. As a result, it is this crop which has been evaluated in greatest detail.

Table 2. NSP content of some legumes (g/kg DM)

	Soya Bean	Peas (white)	Faba Beans	White Lupins
$\alpha$ -Galactosides				
Raffinose	10	5	4	10
Stachyose	47	23	16	53
Verbascose	3	22	34	14
Uronic acids				
Soluble	25	20	24	27
Insoluble	23	12	9	12

Soya beans contain approximately 350g total carbohydrates/kg DM; however, only trace amounts of the soluble carbohydrates in soya beans are monosaccharides such as glucose and arabinose. The larger carbohydrate fraction in soya beans consists of di- and oligosaccharides (Table 2) which are of some considerable nutritional relevance as they are responsible for flatulence following consumption attributable to the low

molecular weight oligosaccharides containing  $\alpha$ -galactosidic and  $\beta$ -fructosidic linkages, namely raffinose and stachyose. Contents of the two are reported as 1 - 9 and 14 - 41g/kg (Hymowitz et al 1972). Interestingly, there are clear differences between different legumes in terms of their NSP content, so legumes cannot be regarded as uniform in this respect.

### 3. NUTRITIONAL IMPLICATIONS

NSPs are of particular importance in that their digestibility is often the lowest among components of diets. Water-insoluble NSP can cause great variations in the dietary energy values between species and diets because of the considerable range in concentrations (10-400g/kg grain) and digestibility (0-0.85).

NSPs in poultry diets have been shown to affect feed intake, increase water intake, beak impaction and vent plugging, the size of the gastro-intestinal tract and the water content of the digestive tract content and excreta. Pigs and poultry do not produce enzymes capable of digesting cellulose, arabinoxylans,  $\beta$ -glucans or pectins.

The plant cell wall is complex and insoluble and its breakdown requires the action of multiple enzyme activities whose effects may not be additive. Considering the complexity of the cell wall it is not surprising that purified enzymes are not as successful in hydrolysis of ANFs as more crude sources. It was considered that  $\beta$ -glucan may also provide an energy source if degraded to glucose monomers. However, low levels of  $\beta$ -glucosidase in the enzyme source, enzyme concentrations and the time frame involved was not sufficient for effective degradation to free glucose (White, et al. 1981). The arguments pertaining to the breakdown of  $\beta$ -glucan to monomers apply equally to pentosans which are probably even more difficult to digest due to their complex structure (Classen & Bedford 1991). In addition it should not be forgotten that the constituent pentose monomers have a lower efficiency of utilization *in vivo* than glucose (Shutte, et al. 1992).

Increased viscosity can cause intestinal bacteria to multiply to an extent which disadvantages the host. Many consider that the stability of the gut microflora ecosystem can be affected by four factors, which are microbial species diversity, nutrient or substrate diversity, colonization diversity and colonization resistance. The consequence of an increased energy supply to the microflora is not only increased microbial growth but also a shift in the bacterial species. Increased bacterial activity in the gastro-intestinal tract leads to increased levels of ammonia, amines, volatile fatty acids, secondary bile acids and bacterial enterotoxins, all of which have a damaging effect on the mucosa of the small intestine.

Soluble  $\beta$ -glucans and arabinoxylans are thought to be responsible for impeding digestion by causing a viscous intestinal environment. Unimpeded movement of enzymes, substrates and products of digestion by diffusion in the gut is essential for rapid digestion. However, as the viscosity of the gut contents increases, the rate of

diffusion decreases, hence reducing growth rates, feed efficiency and the dietary energy value of the diet. Although an increase in intestinal viscosity can cause wet litter problems in poultry, reduced feed throughput and intake which limits the nutrient assimilation rate, it could also be argued that increased viscosity allows more time for digestion by endogenous enzymes. However, the slower rate of feed transit allows for intestinal bacteria to multiply and migrate to the upper reaches of the small intestine. These microflora will compete with the host in the digestion and utilization of starch and protein in the digesta, therefore exacerbating the situation for the chick. Thus unimpeded movement of enzymes, substrates and products by diffusion through the gut is essential for rapid digestion.

NSPs probably also contribute to the antinutritive actions by encapsulating the nutrients inside the endosperm. Digestion of these insoluble ANFs results in improved exposure of starch and protein to digestive enzyme action. However, once soluble, NSPs produce viscous solutions by aggregating into large networks or meshlike structures from the entanglements of large polymers. Thus the antinutritive activity of arabinoxylans and  $\beta$ -glucans in chickens and pigs is not a function of the polymers *per se* but of the intestinal viscosity they create.

In terms of nutrition, the water-soluble NSP fraction is often considered as that which is rapidly solubilized and fermented in the digestive tract of the host. In recent years there has been a growing interest in water soluble NSP since these components are known to have specific physiological effects. Their chemical structures can influence both the rate and extent of digestion and hence can be used to control and optimize nutrient assimilation in different parts of the gastro-intestinal tract (Aman & Graham 1990). Several polysaccharides are considered to have antinutritional properties and therefore the effect of processing and exogenous enzyme supplementation, particularly on NSP, have been of particular interest in recent years. However, even if added exogenous enzymes were able to hydrolyse part of the water-insoluble NSP in the digestive tract, it remains to be seen whether gut bacteria have time to metabolize the end products of enzyme hydrolyses, particularly of diets with low digestibility coefficients. Furthermore, the monomeric pentoses from the complete hydrolysis of, for example, arabinoxylans (arabinose and xylose) are used less efficiently in metabolic pathways than glucose when absorbed by the bird (Schutte, et al. 1992). It would appear that as a preliminary comment, the benefits to be gained from exogenous enzymes are in terms of modification to gut environment through partial hydrolysis of NSP rather than by releasing nutrients associated with them.

## A. Cereals

Since glucans and pentosans comprise the major components of cell walls of barley endosperm it seems very likely that the solubilization of cell walls and breakdown of highly viscous cell components enhances the starch availability for hydrolysing enzymes within the intestinal tract of broiler chickens. This may subsequently increase the productive value of barley (e.g. Hesselman & Aman 1985). Results indicate that at the

end of the small intestine 96-98% of the starch had disappeared when  $\beta$ -glucanase had been included in barley diets, compared with 85-88% when unsupplemented diets were fed.

Research has connected  $\beta$ -glucans to viscosity produced by barley. However total  $\beta$ -glucan may not be the most important factor in determining viscosity characteristics since solubility, molecular weight and structure will ultimately have an effect (Wood, Weisz & Mahn 1991). The solubility of  $\beta$ -glucans is determined not so much by their degree of polymerization but rather by small differences in fine structure which alter the ability of chains to align into relatively stable molecular aggregates (Woodward, Fincher & Stone 1983; Woodward, Phillips & Fincher 1983).

In light of other possible actions of NSP, defining their antinutritive effects solely due to increases in digesta viscosity is probably too simplistic. Viscosity and solubility effects should not however be forgotten when investigating alternative mechanisms. It may be that there is a greater relationship between *in vivo* soluble NSP levels and viscosity of wheat and barley than perhaps originally realized.

Hulless varieties of barley and oats contain 10-15% less crude fibre and were initially thought to resolve problems of the poor nutritive values of these grains by increasing the relative concentration of digestible nutrients. However, this has not proved to be the case and attention focused on the  $\beta$ -glucans and arabinoxylans located predominantly in the endosperm rather than the cellulose in the pericarp and hull.

Determination by Bhatti (1987) of  $\beta$ -glucan in two hulless barley samples (Table 3) has shown higher  $\beta$ -glucan levels and higher acid extract viscosity than that found in hulled barley. Growth depression seen in chicks fed hulled barley can be related to extract viscosity of the barley according to Bhatti, et al. (1991).

Table 3  $\beta$ -glucan levels and acid extract viscosities of hulled and hulless barley

Genotype	Total $\beta$ -glucan g/kg	Soluble $\beta$ -glucan g/kg	Insoluble $\beta$ -glucan g/kg	Viscosity centistokes
Bonanza (hulled) n=8	26.2 $\pm$ 1.4	8.4 $\pm$ 0.5	17.8	7.40
Tupper (hulless) n=5	27.8 $\pm$ 0.7	8.3 $\pm$ 0.7	19.5	9.76
Scout (hulless) n=2	34.2 $\pm$ 1.1	14.5 $\pm$ 0.6	19.7	25.91

From Bhatti (1987)

Older chicks were apparently better able to cope with the higher viscosity induced by feeding barley, although the fact that the higher viscosity remained indicated that

enzyme supplementation may still be required to reduce associated sticky litter conditions (Salih, et al. 1991).

It was originally considered that plant breeding to remove the outer husk in barley to produce hullless cultivars would be associated with a sample of better nutritional value as total crude fibre levels would be reduced. However, the husk is highly cellulosic and lignified; its complexity is such that it is almost inert physiologically and nutritionally. The data in table 4, generated from a study where individual fractions were obtained following milling and then included in diets at the same rate as would be found with the ground whole cereal, demonstrate that the lowest digesta viscosity was associated with the husk in barley and that it was the endosperm with its high levels of water soluble NSPs which gave by far the highest viscosity. Thus conclusions on the suggested nutritional improvement to barley by removing husks and lowering crude fibre were completely erroneous; removing husks would decrease crude fibre but increase water soluble NSPs.

Table 4. Inclusion of various cereal fractions on digesta viscosity in broilers

Inclusion g/kg diet	Component	Viscosity cps	Inclusion g/kg diet	Component	Viscosity cps
500	Whole barley	19	500	Whole wheat	5
600		16	600		9
700		14	700		10
18	Husk	1	65	Bran	6
21		2	78		8
25		2	91		3
364	Endosperm	30	50	Offal	3
436		24	60		2
509		22	70		3
94	Flour	7	385	Flour	7
113		6	462		5
132		7	539		6

Petersen et al (1994)

The use of barley in poultry diets has increased greatly with the availability of enzymes with  $\beta$ -glucanase activity. Extensive literature describes the effects of  $\beta$ -glucanases, which include reducing intestinal viscosity, hydrolysing the  $\beta$ -glucans, releasing encapsulated nutrients to digestive enzymes and generally increasing the absorption of most nutrients in the diet. The extent of improvement can be variable and depends on age of animal, barley grain used (cultivar, ripeness) the amount of barley within the diet and how it is fed, either mash or pelleted. Nevertheless it has been noted that enzyme addition to barley diets consistently improved the performance of broilers and reduced the variability among barley samples.

Finally it should not be forgotten that viscosity associated with cereal NSPs is not the only reason why exogenous enzymes are considered in cereal based diets. Recently it has become clear that maize, which has a comparatively low level of NSP, may have variable nutritional value which may be reduced through enzyme use although regarded as a 'non-viscous' cereal.

## B. Legumes

Non-ruminants do not possess the enzyme ( $\alpha$ -galactosidase) necessary for hydrolysing the  $\alpha$ -galactosidic linkages of raffinose and stachyose. As a result, oligosaccharides enter the lower intestine where they are metabolised by microflora producing gases responsible for nausea, cramps, wet droppings and diarrhoea. In poultry the  $\alpha$ -galactoside family of oligosaccharides has been implicated in reducing soya bean meal true metabolisable energy (TME), fibre digestion and intestinal transit time (Coon *et al.*, 1990).  $\alpha$ -galactosides can also increase the osmotic pressure of the luminal contents.

The large intestine in growing finishing pigs may possess a sufficient population of microbes to degrade non-starch polysaccharides and provide a potential source of energy (lactic acid and volatile fatty acids) for the animal to absorb (even though increased microbial activity will reduce overall digestive efficiency). However, Veldman *et al.* (1993) reported that the presence of  $\alpha$ -galactosides in the diet of piglets caused fluid retention and increased microbial activity which may result in systemic and local effects such as stimulated gut motility, gut wall damage and decreased hydrolysis of dietary constituents resulting in a diminished overall digestion.

Nutritional significance of soya bean meal oligosaccharides remains controversial. Removal of  $\alpha$ -galactosides using ethanol extraction results in improvement in TME of soya bean meal (Coon *et al.*, 1990) but interpretation of these data is confounded by the simultaneous probable extraction of other meal components. In contrast, Angel *et al.* (1988) reported that removal using endogenous soya bean  $\alpha$ -galactosidase failed to produce any beneficial effect on the nutritional value of soya flakes and concluded poor energy utilisation from soya bean meal (toasted-defatted soya flakes) by poultry is not related exclusively to the presence of the oligosaccharides raffinose and stachyose. Veldman *et al.* (1993) observed that the addition of velasse, the residue after evaporation of a 0.8 ethanol extract of soya bean meal generated during the production of soya protein concentrate, had a significant adverse effect on the ileal digestibility of nutrients and resulted in fluid retention and enhanced microbial fermentation in the gut when fed to piglets. However, the addition of an  $\alpha$ -galactosidase to the velasse diet did not overcome these problems.

Irish *et al.* (1995) evaluated the effects of removing the  $\alpha$ -galactosides of soya bean using either ethanol extraction or exogenous  $\alpha$ -galactosidase enzyme ( $\alpha$ -D-galactoside galactohydrolase) with and without invertase ( $\beta$ -fructofuranoside fructohydrolase) on the nutritional value of soya bean meal. It was shown that the performance of broilers and the TME value obtained with adult birds was not improved by removing stachyose and raffinose from soya bean meal using either ethanol extraction or  $\alpha$ -galactosidase; it

was concluded (table 5) that soya bean meal oligosaccharides have little or no anti-nutritional effect. Evidently, more studies are required to provide information on the nutritional consequences of legume NSPs.

Table 5. Performance of broilers and true metabolisable energy (TME) of differently treated soya bean meal.

	Weight Gain (g)	Gain:feed	Coefficient of apparent digestibility	TME (kcal/kg DM)
Soya bean meal	365	0.706	0.91	2960
Ethanol extracted soya bean meal	272	0.622	0.85	2780
Water incubated soya bean meal	343	0.685	0.92	2745
Water + $\alpha$ -galactosidase incubated soya bean meal	345	0.696	0.90	2705

Irish et al 1995

The use of exogenous proteases with soya bean meal has received attention. The study of Rooke et al (1998) found that protease treatment of soya bean meal prior to feeding improved performance of piglets in the first week post weaning (Table 6)

Table 6. Daily gain of piglets in the first week post-weaning fed different diets based on:

	Untreated SBM	Acid Treated SBM	Protease Treated SBM	Skim Milk	Soya Protein Concentrate
Gain (g/day)	95	121	155	141	129

Rooke et al 1998

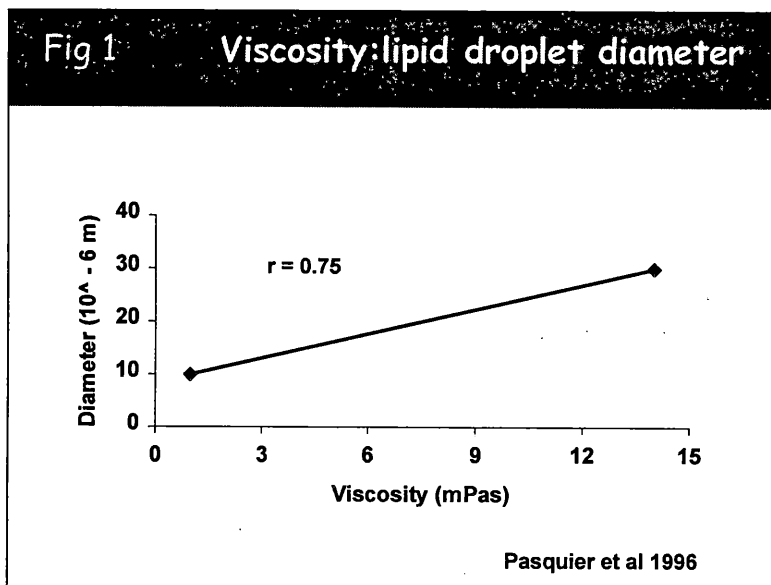
### C. Fats and Oils

It is well-established that pancreatic lipase secretion in young birds and piglets may be a limiting factor in the utilisation of fats and oils. However, addition of lipase to diets is rarely effective as it is emulsification with bile salts which is a major step in fat and oil digestion (e.g. Freeman 1984).

There have been recent studies on the interactions between dietary fats and oils and 'fibre' in terms of digestive efficiency. Interestingly, some studies have been with

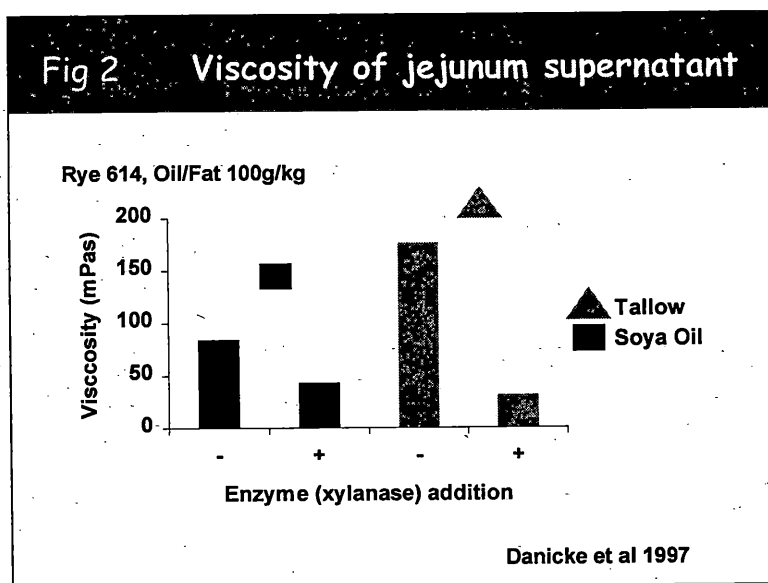
humans in attempts to reduce the post-prandial hyperlipidaemic response in patients prone to obesity; the objectives were to lower lipid uptake through reducing the efficiency of overall lipid digestion. Of course, in the nutrition of pigs and poultry, the objective is the opposite in promoting energy intake by improving fat and oil digestion. Therefore, dietary components leading to reductions in lipid uptake in humans should be interesting in animals so that these reductions can be avoided.

As emulsification is one of the principal stages in fat digestion, the size and the physical properties of the subsequent droplets are important determinants of the degree of binding of pancreatic lipase. The greater the size the smaller the surface area per unit of mass of fat presented and, hence, the lower the efficiency of lipolysis. Recently in an *in vitro* study, Pasquier *et al.* (1996) examined the role of different soluble dietary 'fibres' of varying intrinsic viscosity on the degree of emulsification and lipolysis of tri-acylglycerides. A clear correlation between viscosity and droplet size was suggested (figure 1) associated subsequently with the degree of tri-acylglyceride lipolysis.



Problems of increased viscosity have been described earlier in this paper. In view of the possible effects of soluble NSPs on lipolysis, studies with poultry have been undertaken examining exogenous enzyme addition and fat digestion (e.g. Danicke *et al.*, 1997a,b; Langhout *et al.*, 1997). The general conclusions were that the presence of viscous NSPs within the digesta will compromise digestion and absorption of dietary fats as a consequence of lowered degree of emulsification, reduced activity of pancreatic lipase and decreased micelle formation. Thus problems are more evident the greater the degree of saturation of dietary fat (figure 2) and will also be influenced by molecular weight and size of the NSP fraction which will influence their solubility. Gastro-intestinal micro flora promoted by increasing NSP concentration may also be responsible for bile salt deconjugation which will reduce digestive efficiency of fats still further.

It should be noted, however, that the studies by both Danicke *et al.* (1997a,b) and Lanhgout *et al.* (1997) employed diets that were unusual (high levels of rye and / or high levels of saturated fats). Results from more 'practical' assessments are necessary to establish the quantitative effects these responses might have on nutritional value of fats and oils.



#### 4. CONCLUSIONS

Because of the very large number of published reports on exogenous enzymes, it is impossible to provide a synopsis of each and every one of them; as most trials reported were conducted under specific conditions, comparisons become difficult. There have, however, been recent attempts to bring together all papers in a predictive model in an effort to determine the more important input variables and responses achieved (Rosen, 2002); in addition to moving to a more structured approach to assessing efficacy of exogenous enzyme use, such a development also has the advantage of identifying both positive and negative aspects of trial design which should guide work in the future.

A significant issue with respect to exogenous enzyme use is that there are, for example, possibly hundreds of  $\beta$ -glucanases from tens if not hundreds of prokaryotic species that can reduce the viscosity associated with  $\beta$ -glucan. Selection of which enzymes might be efficacious for adding to non-ruminant diets is a taxing scientific problem and one that is also limited by registration requirements.

Enzymes, as all proteins, are susceptible to heat denaturation and therefore inactivation. There is increased interest in high temperature feed processing in Europe mainly for hygiene reasons. Thus selection of enzymes that are inherently heat stable and application of stabilizing technologies by feed compounders can enhance the stability of the product. Similar issues of stability apply to high moisture levels employed in pelleting through steam injection.

In the future, genetic engineering may be able to introduce more desirable qualities for feed enzymes. Eventually the appropriate enzymes may be produced by transgenic animals, which means self-digestion may become an integral characteristic of feed ingredients. Finally, plant breeding with molecular techniques to develop cultivars with their own complement of enzymes which will be expressed only when consumed may be a future possibility. However, these remain particularly controversial issues in the current climate.

Ultimately for feed manufacturers, the feasibility of any particular application (assuming that appropriate registration issues have been addressed successfully) will depend on the magnitude of the animal response compared with the cost of the enzyme product.

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# FIBER NUTRITION OF SOWS

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## INTRODUCTION

Economic and societal pressures faced by modern pork production systems have increased the interest of pork producers in diets with elevated concentrations of fiber. Small margins of profit force producers to seek methods of increasing output and/or decreasing costs of production. Several reports in the scientific literature (Grieshop et al., 2001) suggest improved reproduction in sows fed diets high in fiber compared to the standard grain-soybean meal based diets used in most of the U.S. swine industry. In addition to economic forces, many consumers and pork producers are questioning if a grain-soybean meal based diet fed in restricted amounts compromises the welfare of gestating sows. Increasing the amount of feed offered to sows each day may decrease undesirable behaviors and improve the level of satiation experienced by the sow. One way to increase the quantity of feed offered to pregnant sows without encouraging excessive fat deposition is to include high levels of fiber in the diet. Finally, steady increases in the world's human population will increase the competition between pigs and people for grains with high nutrient density (CAST, 1999). Inclusion of fibrous feed ingredients with relatively lower nutrient density in some swine diets will decrease this competition for valuable grains. These reasons lead our research group at the University of Minnesota to study the utility of feeding high fiber diets to swine, specifically pregnant sows.

## CHARACTERIZATION OF FIBER

The most widely accepted definition of fiber states that fiber is the sum of lignin and polysaccharides that are not digested by endogenous secretions of the digestive tract (Trowell et al., 1976). This definition segregates dietary polysaccharides into starch and non-starch polysaccharides (NSP) since starch is almost completely digested in the mammalian digestive tract. NSP commonly is used interchangeably with the term, fiber, when referring to fiber in diets. On the surface, the definition proposed by Trowell et al. (1976) seems simple and easily applied to practical swine nutrition. In reality, the definition and quantification of fiber in swine diets is difficult due to the complexity and diversity of polysaccharides involved (Low, 1993; Grieshop et al., 2001).

Weende Crude Fiber, Van Soest Fiber, and Total Dietary Fiber (TDF) are the three predominant methods of fiber characterization that can be applied to swine diets. A more thorough description of these methods is presented in recent reviews by Low (1993), Prosky et al. (1985), and Moore and Hatfield (1994). Each method has its strengths and weaknesses. A comparison of the three methods of fiber characterization in selected fibrous feed ingredients is shown in Table 1.

Table 1. Corn, soybean meal and other fibrous feedstuffs fed to livestock<sup>a</sup>

Feed Ingredient	CF, %	NDF, %	ADF, %	TDF, %	SDF, %	IDF, %
Corn	2.6	9.0	3.0	6.4	1.7	4.7
SBM 44% CP	7.0	13.3	9.4	33.1	1.6	31.5
SBM 47% CP	3.0	8.9	5.4	27.6	1.4	26.2
Alfalfa	26.2	45.0	35.0	56.7	4.2	52.4
Oat Bran	-	19.2	-	15.8	7.5	8.3
DDGS	9.9	44.0	18.0	42.9	0.7	42.2
Oat Straw	40.5	70.0	47.0	76.6	2.2	74.4
Soybean hulls	40.1	67.0	50.0	83.9	8.4	75.5
Wheat Straw	41.6	85.0	54.0	71.5	0.5	71.0
Corn Stalk	34.4	67.0	39.0	77.3	2.9	74.4
S. Beet Pulp	19.8	54.0	33.0	65.6	11.7	53.9
Potato Pulp	-	-	-	33.3	11.0	22.3

<sup>a</sup>Sources: NRC (1998); NRC (1988); Dale (1998); and U of M laboratory analysis.

Crude fiber analysis involves sequential extractions of feed with petroleum ether, dilute acid and dilute alkali to yield a residue called crude fiber (Close, 1993). While this approach is simple and quick, it underestimates the concentrations of cellulose, hemicellulose, and other soluble NSP (Van Soest and McQueen, 1973). Hemicellulose and soluble NSP are important components of feed that influence nutrient utilization and metabolism. Detergent methods of fiber analysis were developed by Van Soest (1963a,b) which improved on the Weende approach. The detergent method yields neutral detergent fiber (NDF) and acid detergent fiber (ADF) fractions. NDF contains lignin, cellulose and variable portions of hemicellulose. Water-soluble NSP fractions are lost in the determination. ADF is a reliable measure of cellulose and lignin.

The inability of existing systems to quantify water-soluble NSP led to the development of the Total Dietary Fiber (TDF) system of fiber analysis (Prosky et al., 1988). In this approach, soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) are determined gravimetrically and summed to derive TDF. This approach is used primarily for evaluation of human foods but can be applied to feedstuffs for swine. TDF is most like NDF of the Van Soest analytical method (Table 1). The primary shortcoming with the TDF (and other gravimetric approaches such as crude fiber and Van Soest fiber) is that remaining residues are assumed to be composed entirely of fibrous compounds. This is not entirely true because lignin and starch resistant to enzymatic digestion are present which inflates estimates of fiber. In addition, SDF from one feedstuff does not have the same composition as SDF from another source. In other words, we are not dealing with a consistent, homogeneous compound or mix of compounds when we talk about SDF or IDF or ADF or NDF. Englyst and Cummings (1988) used a procedure to quantify the amounts of constituent sugars in NSP by gas-liquid chromatography and colorimetry then derived total NSP concentration via summation. This procedure is more expensive and time consuming than gravimetric procedures.

place of the more traditional crude fiber. However, they provide no recommended levels of NSP for swine diets. We believe that TDF is a more appropriate measure of dietary NSP for swine because it accounts for water-soluble NSP such as pectins,  $\beta$ -glucans, fructans and other soluble sugars. These compounds have important effects on the digestion and metabolism of nutrients in swine. Unfortunately, there is a dearth of information available on the SDF and ISF content of common feedstuffs used in swine diets.

## NUTRITIONAL EFFECTS OF FIBER

The quantity and character of dietary NSP greatly influences the site and degree to which dietary polysaccharides are digested. It is difficult to describe with certainty the effects of various fiber components (SDF or IDF) on digestibility because they are not a homogenous substance. Fibrous feeds may contain predominantly one type of fiber or another type but they are not pure. Consequently, conclusions are drawn from a given fiber source that is predominantly one type of fiber and those conclusions are used to generalize the effects of that fiber component.

As mentioned previously, starch is almost completely digested (90 to 100%) by the time digesta reaches the ileal-cecal junction (Bach Knudsen and Hansen, 1991; LeGoff and Noblet, 2001). In contrast, lignin is not digested by pigs nor is there any significant fermentation by resident microbes in the gut (Graham et al., 1986; Shi and Noblet, 1993). In addition to being indigestible, lignin influences the digestibility of other fibrous components of the diet. As a plant matures, cellulose becomes intertwined with lignin to increase the rigidity of the plant structure. In this process, cellulose becomes less accessible to microbes in the hindgut, which depresses the rate and extent of fermentation. Diet digestibility is inversely proportional to lignin concentration. Pectins, fructans,  $\beta$ -glucans, and other components of SDF increase viscosity of the digesta (Mosenthin et al., 2001; Noblet and LeGoff, 2001). Increased viscosity in the small intestine might slow gut transit time due to suppressed intestinal contractions (Cherbut et al., 1990) which in turn leads to less mixing of dietary components with endogenous digestive enzymes. The end result is that SDF may interfere with complete digestion of dietary components (fibrous and non-fibrous) in the small intestine. However, the swelling associated with increased viscosity creates a much greater surface area for microbial attack in the hindgut. This partly explains the relatively high total tract digestibility of soluble fiber (Noblet and LeGoff, 2001).

Insoluble dietary fiber is digested primarily in the hindgut as a result of fermentation (Noblet and Shi, 1993; Shi and Noblet, 1993). Pigs do not secrete enzymes in the small intestine that attack components of IDF so they pass through relatively untouched to the large intestine (Shi and Noblet, 1993; Varel and Yen, 1997). Insoluble dietary fiber can negatively affect total tract digestibility of dietary nitrogen (Shi and Noblet, 1993; LeGoff and Noblet, 2001) and ether extract (LeGoff and Noblet, 2001). Pigs fed high fiber diets have proportionally heavier gastro-intestinal tracts than pigs fed low fiber diets which contributes to slight increases in maintenance energy requirements (Rijnen et al., 2001; Yen et al., 2001). Fermentation of NSP in the hindgut of pigs yields short chain

fatty acids (SCFA) and lactic acid (Bach Knudsen and Jorgensen, 2001). This hindgut fermentation can generate 17% of the total digestible energy derived from the diet in growing pigs and 25% in sows (Shi and Noblet, 1993). These end-products of fermentation can supply 24 to 30% of the energy needs for growing pigs (Rerat et al., 1987; Yen et al., 1991). In sows, the contribution to daily energy requirements is likely to be greater than that for growing pigs because of the sow's greater ability to digest fibrous feed ingredients (Table 2).

Total tract digestibility of NSP increases as the pig matures (Cunningham et al., 1962). For most types of NSP, sows possess higher digestibility coefficients than growing pigs (Fernandez et al., 1986; Noblet and Shi, 1993; LeGoff and Noblet, 2001). The improvement with age is particularly noticeable with feedstuffs that are high in IDF, which is digested mainly in the hindgut (LeGoff and Noblet, 2001; Table 2). Improved digestibility of NSP with age results from a more voluminous large intestine and cecum (Kass et al., 1980; Pekas, 1991) that contain a more extensive microbial population and fermentation (Yen, 2001). Furthermore, sows generally receive a much smaller quantity of feed relative to their body size compared with growing pigs. This practice allows slower transit time of digesta and greater contact of endogenous enzymes and microbial populations with feed in the gut which should improve digestibility.

Table 2. Digestibility coefficients for energy in growing pigs and sows<sup>a</sup>

Ingredient	Crude fiber, %	Digestibility of energy	
		Growing pigs	Sows
Wheat	2.5	.875	.892
Corn	2.8	.889	.916
Barley	4.6	.826	.835
Soybeans	5.6	.732	.817
Lupins	17.5	.768	.849
Soybean meal	7.2	.845	.894
Sunflower meal (dehulled)	10.3	.737	.789
Corn gluten feed	7.3	.686	.755
Wheat bran	10.0	.585	.646
Sugar beet pulp	21.0	.698	.764
Soybean hulls	39.1	.473	.712
Sunflower hulls	57.0	.284	.341

<sup>a</sup>After Noblet and LeGoff, 2001.

In vivo digestibility of fibrous feed ingredients is usually determined in young growing pigs. However, it is clear that sows have a greater capacity to extract energy from fibrous feedstuffs compared with growing pigs. So, one must carefully extrapolate digestibility data determined in growing pigs to sows. Noblet and LeGoff (2001) reported a regression equation that predicts DE of diets for sows from DE determined in growing pigs. Unfortunately, this equation underestimates energy value of feeds for sows, particularly in low energy feeds or ingredients. Alternatively, the French research group (Noblet and Shi, 1993; Noblet and LeGoff, 2001) have proposed a specific set of equations to predict energy content of feeds for sows from chemical analysis of the feed.

These equations may have some utility. Some require starch and sugar concentrations of the feeds as inputs. We do not routinely analyze feed ingredients for starch and sugar content in the U.S.

The University of Minnesota's Swine Nutrition Group is interested in studying the effects of dietary NSP on reproductive performance of sows. While there are many effects of dietary NSP on sows, we are interested specifically in effects of fiber type on litter size. There are several scientific reports of the effects of various fiber sources on litter size in swine (see below). However from these studies, one cannot establish sound recommendations on the type or level of dietary fiber to include in diets for reproducing sows. We set out to determine the effects of SDF, IDF or a SDF/IDF combination on reproductive performance of sows. From the feedstuffs described in Table 1, we chose oat bran as a source of SDF, wheat straw as a source of IDF, and sugar beet pulp because it contains high levels of SDF and IDF. These ingredients were selected because of their fiber fractions realizing that they are not necessarily very practical feed ingredients for use in commercial pork production systems. Experimental diets were fed to primiparous and multiparous sows throughout gestation (Table 3).

Table 3. Composition and analysis of experimental diets

Ingredient	Control	SDF	IDF	SDF/IDF
	%			
Corn	83.26	54	71.72	68.36
Soybean meal	13.4	8.5	13.36	12.68
Oat bran	.....	34.32	.....	.....
Wheat straw	.....	.....	11.64	.....
Sugar beet pulp	.....	.....	.....	15.92
Dicalcium phosphate	1.47	1.35	1.63	1.65
Limestone	1.07	1.03	0.85	0.59
Salt	0.40	0.40	0.40	0.40
Vitamin premix	0.30	0.30	0.30	0.30
Mineral premix	0.10	0.10	0.10	0.10
TOTAL	100.00	100.00	100.00	100.00
<i>Calculated values</i>				
ME, Mcal/kg	3.300	3.267	3.080	3.164
Crude protein, %	13.2	13.6	12.6	13.0
NDF, %	9.18	12.53	17.97	15.65
ADF, %	3.05	1.97	9.01	7.37
<i>Analyzed values</i>				
ME, Mcal/kg	3.210	3.337	3.147	2.998
SF, %	1.20	3.02	1.11	2.32
ISF, %	9.78	10.11	17.86	16.08
TDF, %	10.98	13.13	18.97	18.40

Energy intake during the early post-mating period can have important effects on embryo survival and potentially litter size at birth. Consequently, we determined the energy and nitrogen digestibility of the experimental diets in sows. These data will allow us to ensure equal intake of digestible energy and nitrogen for sows fed the four experimental

diets during gestation in a large performance trial. This approach will allow us to determine the effects of fiber type/source independent of energy intake.

Digestibility of energy and nitrogen for the diet high in SDF was similar to that of the control diet (Table 4). Apparent digestibility of energy and nitrogen was depressed by the addition of IDF as wheat straw. Energy digestibility of the SDF/IDF diet based on sugar beet pulp was intermediate likely due to its combination of highly digestible SDF and IDF that has a lower degree of digestibility. Apparent digestibility of N in the SDF/IDF diet was depressed compared to SDF and control diets. Fecal dry matter output was highest for the IDF diet which demonstrated the lowest digestibility. This observation is significant as one considers the environmental impacts of increased quantities of manure that are generated when feeding some high fiber diets. An important observation is that diets containing very high levels of fibrous feed ingredients can be just as digestible as high starch diets. The degree of digestibility is dependent on the character of fiber.

Table 4. Effect of diets high in SDF and IDF on energy and N digestibility

Item	Control	SDF	IDF	SDF/IDF
Feed intake, g/d	1826	1870	1961	1915
Sow wt. gain, g/d	315	302	313	334
Fecal DM output, g/d	180	175.3	346.2	207.9
Urine output, g/d	5707	7700	4754	7006
App. digestibility of GE, %	87.9	89.3	82.7	86.8
App. digestibility of N, %	86.1	86.2	82.8	82.8

### EFFECTS OF FIBER ON REPRODUCTION

Gestation is the most logical phase of the reproductive cycle to feed fibrous diets to sows because nutrient requirements of pregnant sows are less than growing pigs and lactating sows so diets of relatively low digestibility are not suitable for these classes of pigs. The sow's ability to consume commercial diets far exceeds her nutrient needs during pregnancy. Many studies have reported improvements in reproductive performance of sows fed diets high in NSP (Grieshop et al., 2001). The magnitude of improvement is quite variable and in some cases researchers reported no improvement or a depression in performance due to dietary NSP addition. Grieshop et al. (2001) summarized 20 studies reported in the literature that investigated effects of high fiber diets on reproductive performance of sows. Thirteen of 19 studies that reported data on litter size found an increase in litter size when sows received a diet high in NSP during the previous gestation. Six studies reported no difference or a depression in litter size. The magnitude of positive responses ranged from .1 to 2.3 pigs born live per litter. The overall weighted mean response for all 19 studies was .4 pigs. They also found a slight increase in voluntary feed intake during lactation (5.4 vs 5.6 kg/d) when sows received high fiber diets during the previous gestation. It is not clear if this small increase is directly attributable to dietary NSP or to the smaller gestation weight gain of sows fed fibrous

diets during pregnancy. Fibrous diets during gestation improved sow longevity in four out of eight studies that reported such data.

The recurring improvement in litter size due to elevated dietary NSP seems real but the inconsistent nature of the response makes reliable recommendations difficult. Some attribute of high fiber diets seems to favor improved reproduction. We surmised two possible explanations for the positive reports in the literature. Firstly, sows fed the high fiber diets during early pregnancy may have consumed less energy than control sows which improved embryo survival in early pregnancy. Secondly, dietary NSP may have elicited improved sensitivity of peripheral tissues to insulin and sustained postprandial secretion of insulin which improved ovulation rate and ultimately litter size at birth.

Dietary energy intake immediately after mating can influence embryo survival. Jindal et al. (1996) reported improved embryo survival when pre-mating high level feeding (flushing) was discontinued within one day after mating. High feeding levels post-mating increase clearance rate of progesterone which is detrimental to synchronous development of embryos (Jindal et al., 1996; 1997). Asynchronous embryo development encourages elevated embryo mortality. Positive responses in litter size to dietary NSP may be attributable to the energy dilution that occurs when diets contain elevated levels of NSP. Sows fed the fibrous diets may have consumed less energy during the critical early stages of pregnancy which enhanced embryo survival and ultimately, increased litter size at birth. Unfortunately, this theory cannot be tested with studies reported in the literature because those studies did not adequately describe or evaluate the energy content of the diets high in NSP.

Dietary NSP can influence sensitivity of peripheral tissue to endogenous insulin and alter secretion patterns of insulin. Several authors have reported increased sensitivity of peripheral tissues to insulin in humans that suffer from non-insulin dependent diabetes when their diet contains elevated levels of dietary NSP (Hjollund et al., 1983; Karlstrom et al., 1984; Landin et al., 1992). Others have reported prolonged insulin secretion after a meal when guar gum, a source of soluble dietary fiber, is added to the diet of healthy men (Nestler et al., 1988). It appears that SDF compared with other fiber fractions is most effective in influencing insulin sensitivity and secretion. Insulin can have gonadotrophic effects on the ovary (Poretsky and Kalin, 1987). Elevated concentrations of insulin in blood enhance ovulation rate (Cox et al., 1987) and subsequent litter size (Ramirez et al., 1993). One may theorize that elevated dietary fiber consumed during the follicular phase of the estrous cycle increases sensitivity of the ovaries to circulating insulin which results in higher ovulation rates. Higher ovulation rates could lead to larger litter size at farrowing. Currently, we are conducting experiments to evaluate this hypothesis.

An obvious question of practicing swine nutritionists is "What level of dietary NSP intake is required to elicit improvements in litter size?" The handling problems associated with high fiber diets encourages producers to use the lowest concentration of dietary fiber necessary to achieve the desired effect. To address this question, we selected six experiments from the literature that reported positive effects of high fiber diets on litter size. We calculated the magnitude of improvement in litter size attributable

to diets high in NSP and ranked these responses according to NDF intake (Table 5). NDF intake of experimental sows was not reported in many studies so we used the reported diet composition and tabular NDF concentration of ingredients to calculate daily NDF intake of sows.

Table 5. Relationship of estimated NDF intake of gestating sows to litter size at birth

NDF intake, g/d	Difference from control		Reference
	Total pigs/litter	Live pigs/litter	
520	.80	.80	Hagen et al., 1987
526	.70	.70	Danielson and Noonan, 1975
640	--	1.00	Pollman et al., 1981
660	.55	-.02	Danielson and Noonan, 1975
880	.80	.80	Hagen et al., 1987
922	.56	.49	Danielson and Noonan, 1975
970	1.10	1.10	Honeyman and Zimmerman, 1990
1010	.80	.50	Danielson and Noonan, 1975
1030	.60	.60	Holzgrafe et al., 1986
1110	.60	.50	Honeyman and Zimmerman, 1990
1240	-.99	-1.10	Hagen et al., 1987
1860	--	1.20	Matte et al., 1994
2020	--	.70	Matte et al., 1994

There appears to be no particular level of NDF intake that results in a maximal litter size response. NDF intakes of 520, 880, or 1010 g/d elicit the same numerical response. One conclusion is that all the NDF intakes in this sample exceeded that required to elicit a litter size response. Consequently, one might recommend an NDF intake of something less than 520 g/d but how much less? Reese (1997) recommended that sows consume 450 g NDF/d if fed alfalfa haylage, alfalfa meal or alfalfa hay; 515 g NDF/d if fed oat hulls; 380 g NDF/d if fed corn gluten feed; or 368 g NDF/d if fed wheat straw. An extensive commitment of time and resources would be required to establish ingredient specific intakes of NSP to elicit a litter size response. Currently, no research group has embarked on such a journey.

One potential explanation for the ingredient-specific recommendations of Reese (1997) may be related to the method of fiber characterization. As described previously, the NDF fraction does not include gums, pectins, glucans and other soluble fiber fractions. These soluble fractions may influence sows' reproductive responses to dietary NSP. This would argue that fiber characterization based on the TDF system would better describe the physiological effects of dietary fiber. We are currently summarizing an experiment to evaluate the reproductive responses of sows fed diets with differing levels of SDF and IDF during gestation.

#### SOW WELFARE AND FIBER

Gestating sows allowed free access to feed will consume nutrients far in excess of their needs for maintenance, pregnancy, and maternal growth. Excess nutrient intake during pregnancy is detrimental to sow performance and longevity (Dourmad et al., 1994).

Consequently, pork producers must limit nutrient intake of pregnant sows to ensure optimal biological performance. The easiest and most common method of nutrient restriction in commercial pork production is to decrease the quantity of feed offered to the sow. However, feed restriction increases the occurrence of undesirable stereotypic behaviors in gilts (Appleby and Lawrence, 1987). The occurrence of undesirable stereotypic behaviors is often used to measure welfare of sows. McGlone and Fullwood (2001) have questioned if a high occurrence of stereotypic behaviors truly reflects compromised welfare of the sow. In contrast, many other researchers and the general public believe stereotypies to be an outward sign of compromised sow welfare due to boredom and frustration so researchers have focused on the effect of high fiber diets on stereotypic behaviors and welfare of sows.

In many studies, diets high in NSP significantly reduced occurrence of stereotypic behaviors in pregnant sows (Robert et al., 1993; Ramonet et al., 1999; Bergeron et al., 2000). In contrast, McGlone and Fullwood (2001) found no effect of a pelleted diet containing 25% sugar beet pulp on stereotypic behaviors. However, pelleting may have altered the character of dietary NSP such that beneficial effects were not realized. Bergeron et al. (2000) reported that high daily intake of a nutrient dense diet was most effective at minimizing stereotypic behaviors. This suggests that sows expressing stereotypic behaviors are frustrated and have an elevated feeding motivation. Diets high in NSP can reduce feeding motivation as indicated in operant conditioning tests with sows and this response differs according to the source of fiber (Robert et al., 1997). However, others have reported no effect of dietary fiber on feeding motivation (Bergeron et al., 2000; Ramonet et al., 2000).

If fibrous diets do decrease stereotypic behaviors and feeding motivation, one would expect sows fed high fiber diets to demonstrate less evidence of stress. McGlone and Fullwood (2001) used several physiological measures to assess stress in pregnant gilts fed a sorghum-soybean meal diet compared with others fed the control diet with 25% sugar beet pulp. These authors concluded that diet had no influence on the stress level of gilts housed in gestation stalls.

The apparent reductions in stereotypic behaviors when diets high in NSP are fed may be related to increased time spent feeding. Several authors have reported that sows require more time to consume their daily feed allowance when diets contain elevated NSP (Brouns et al., 1997; Robert et al., 1997; Ramonet et al., 1999). Brouns et al. (1997) suggests that gastric distention is an important factor in regulating the speed with which sows can consume their daily allotment of feed and their degree of satiation. If sows spend more time each day consuming feed, there is less time available for other activities such as stereotypic behaviors. Increased feeding time may create problems if an electronic feeding station is used to feed sows. The longer feeding time may require a lower stocking rate on each feeding station.

Increased satiation of sows fed diets high in NSP may relate to metabolic factors. Brouns et al. (1994) fed diets high in sugar beet pulp or wheat bran to determine their effects on post-prandial concentrations of insulin, glucose, and acetate in blood. They concluded

that elevated acetate levels and more uniform levels of insulin and glucose may have maintained satiety for a longer period after the meal when compared to sows fed a high starch diet.

The balance of evidence suggests that diets high in NSP will reduce stereotypic behaviors in sows and increase their level of satiety. Currently, we assume that a decline in stereotypic behaviors equate to an improvement in welfare of the sow. The minimum level of NSP required in the diet to achieve these positive effects has not been elucidated. Ramonet et al. (1999) observed marked effects are realized with diets containing greater than 12% crude fiber. Meunier-Salaun (2001) suggested that crude fiber concentrations of 20% are most effective in reducing stereotypic behaviors. Character of the fiber and method of fiber analysis will influence recommendations on the appropriate level of dietary fiber to include for optimal improvements in sow welfare.

### OTHER ISSUES TO CONSIDER

*Handling high fiber diets.* Modern pork production systems depend almost exclusively on mechanical means for feed mixing and conveyance. Bulk bins, chutes, conventional augers, centerless augers, and cable delivery systems are designed to accommodate diets composed of cereal grains and protein concentrate. Addition of bulky, fibrous ingredients to the diet will dramatically change the handling characteristics of the final feed. Reproductive and welfare benefits will have to be realized before commercial producers will retrofit existing feed delivery systems to allow use of fibrous diets.

*Manure management.* Increasing fiber concentration of diets will increase the volume of manure generated by sows (Table 4). The increased volume of manure will increase the rate at which manure storage structures are filled. This may require more frequent removal and spreading of manure or larger capacity manure storage structures. In addition, alterations in composition of the manure may influence the microbial environment in the manure storage structure which might change odor generation and accumulation of solid residues in the structure.

### SUMMARY

There are many reasons not to include high levels of fibrous feed ingredients in diets for gestating sows. The negative effects include decreased digestibility of nutrients with some fibrous ingredients; increased volume of manure generated; increased potential for sludge buildup in liquid manure handling systems; and difficulty of handling bulky diets in mechanical feed manufacturing and conveying systems. However, there are two important reasons to consider increasing the proportion of fibrous ingredients in gestating sow diets. First, reports in the scientific literature suggest that many different sources of dietary fiber will improve litter size. Second, the balance of evidence indicates that limited pregnant sows experience improved satiety and comfort when fed diets high in fiber. Economic pressures drive pork producers to consider increasing dietary fiber so that improved reproductive performance can be captured. Current societal pressures that may someday evolve into societal mandates and producers' desire to constantly improve care

of their sows encourages us to embrace the second benefit and seek ways to overcome the challenges of high fiber diets.

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# INULIN IN SWINE DIETS

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## INTRODUCTION

Carbohydrates are an important component in the diets of many animal species and often constitute a major part of the diet. Up to 80% of the dietary energy in the animal diet can be derived from carbohydrates. Lloyd et al. (1978) indicated that it is perhaps ironic, then, that when the composition of feeds is reported, carbohydrate is often calculated as the residual after fat, nitrogen (protein), and ash have been determined. The role and function of the simple sugars in animal nutrition and metabolism have long been recognized, but only recently has attention been given to more complex carbohydrates such as oligosaccharides, their importance in the diet, and their impact on nutrition, metabolism and health.

## SOLUBLE, FERMENTABLE DIETARY FIBER

Oligosaccharides are naturally occurring carbohydrates that are commonly found in plants. Their chemical and physical properties may vary as a function of structure which can be linear or branched, linkages can be  $\alpha$  or  $\beta$ , and the number and type of monomers can also vary. Fructan is a general name used for any carbohydrates in which one or more fructosyl-fructose link constitutes the majority of osidic bonds. Fructans are linear or branched fructose (oligo) polymers, which are either  $\beta$ -(2-1) linked inulins or  $\beta$ -2,6 linked levans. Inulin has been defined as a polydisperse carbohydrate material consisting mainly of plant origin, whereas some fungi and many bacteria are the major producers of levans (Fuchs, 1991). These carbohydrates can be classified as non-digestible carbohydrates (NDOs) because the  $\beta$ -linkages between fructose monomers in Fructo-oligosaccharides (FOS) and trans-galacto-oligosaccharides (TOS) cannot be hydrolyzed by enzymes of endogenous origin (Oku et al., 1984). Thus inulin can scarcely be hydrolyzed by gastric acid, digestion by human and monogastric animal digestive enzymes does not occur, nor absorption of intact inulin. As a consequence, NDOs are quantitatively available as substrates for the gastrointestinal microflora (Houdijk et al. 1998; Jenkins et al., 1999) where it is fermented by endogenous bacteria. These characteristics, (non-digestible carbohydrate from plants that reaches the colon largely intact and evokes physiological functions) qualifies inulin as dietary fibre and falls under the sub-category of soluble fibre (Roberfroid and Delzenne, 1998). Inulin exerts a preferential stimulatory effect on the numbers of beneficial genus *Bifidobacterium*, while maintaining populations of potential pathogens (*E. coli*, *Clostridium perfringens*) at relatively low levels in the large intestine. In addition to these benefits, supplementing diets with NDO increases the densities of lactic acid producing bacteria, enhanced enteric and systemic immune functions, increased energy and nutrient availability, reduced risk of carcinogenesis and improved levels and profiles of serum lipids (Buddington, 2001).

## SOURCES OF INULIN AND OLIGOFRACTOSE

Fructan-containing plant species are found in a number of mono – and dicotyledonous families such as Liliaceae, Amaryllidaceae, Gramineae, and Composite. Illustration and natural occurrence of inulin is presented in Table 1. The aerial parts of many Gramineae contain high fructan levels, particularly of young seedlings (up to 70% of their dry matter), grasses, and cereals that do not lend themselves to industrial extraction and processing of fructans (Roberfroid and Delzenne (1998). Jerusalem artichoke and chicory are the two main sources commonly used by the Food Industry to produce inulin.

**Table 1: Illustration and natural occurrence of inulin**

Source	Inulin (%)	Scientific Name	Reference:
Jerusalem Artichoke	16 – 20	Heliantus tuberosus	Suzuki & Cucliffe, 1989
Chicory roots	15 – 20	Cichorium intybus	Douglas & Poll, 1986
Dandelion	12 – 15	Taraxacum officinale	Roberfroid et. al. 1993
Garlic	9 – 16	Allium Sativum	Darbyshire and Henry, 1981
Onions	1.1 – 7.5	Allium cepa	Suzuki & Cucliffe, 1989
Asparagus shoot	2 – 3	Asparagus officinalis	Roberfroid et. al. 1993
Wheat	1 – 4	Triticum asetivum	Roberfroid et. al. 1993
Rye	0.5 - 1	Secale cereale	Asami et al. 1989

## PHYSIOLOGICAL EFFECTS OF INULIN IN THE GASTROINTESTINAL TRACT

### 1. *Production of Short-Chain Carboxylic Acids and Related Effects.*

During their passage through the gastrointestinal tract, inulin-type fructans, classified as nondigestible oligosaccharides, never produce fructose (Oku et al. 1984). Rather, their colonic fermentation produces short chain carboxylic acids and lactate plus gases as products of their digestion. In doing so, only part of the energy of these dietary carbohydrates is salvaged, and consequently they can be classified as low-energy feed ingredients. Roberfroid and Delzenne (1998) concluded that supplementing diet with inulin-type fructans decreases the cecal pH and increases the size of the cecal pool of short-chain carboxylic acids, with acetate being the primary acid followed by butyrate and propionate. In this way inulin creates an environment that may be antagonistic to the growth of pathogens and putrefactive bacteria. It is estimated that possibly related to this increase in the pool of short chain carboxylic acids is the effect of inulin-type fructans on the intestinal tissue, leading to hyperplasia of the mucosa and increased wall thickness both in the small intestine (Oku et al. 1984) and in the cecum (Campbell et al. 1997). The resulting increased thickness of the intestinal wall will reduce the risk of bacterial translocation. Part of the produced short chain carboxylic acids (mainly acetate and propionate) will be absorbed into the bloodstream. There the volatile fatty acids can be used can either be used as fuel or give rise to specific effects as summarized in Table 2.

## 2. Effect on Mineral Absorption.

The nondigestible carbohydrates have regularly been accused of causing an impairment in the small intestinal absorption of minerals because of their binding/sequestering effect (Persson et al. 1991). Sunvold et al. (1995) indicated that nondigestible carbohydrates per se do affect mineral absorption or mineral balance, an affect that is more likely to be due to the presence of phytate or other mineral-complexing agents (Sandstead, 1992). However, the minerals that are bound/sequestered and, consequently, not absorbed in the small intestine reach the colon, where they may be released from the carbohydrate matrix and absorbed. Furthermore, Thompson et al. (1991) and Younes et al. (1996) concluded that a high concentration of short-chain carboxylic acids resulting from the colonic fermentation of the non-digestible carbohydrates facilitates the colonic adsorption of minerals, particularly  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . In addition, the presence of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in the colon may have important implications such as, help maintain colonic health by controlling rate of cell turnover (Lipkin and Newmark, 1985) and a high concentration of  $\text{Ca}^{2+}$  in the colonic content may lead to the formation of insoluble bile or fatty acid salts, thus reducing the likely damaging activity of bile or fatty acids on colonic cells (Wargowich and Newmark, 1984). Fructans may also improve mineral absorption and mineral balance because of an osmotic effect that transfers water into the large intestine (Sakata, 1997), thus allowing these minerals to become more soluble. In addition, because fructans are extensively fermented in the large intestine, they cause acidification of the colonic content and consequently raise the concentration of ionized minerals, in particular  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , a condition that favors passive diffusion (Younes et al. 1995). Table 2 indicates some of the colonic and systemic physiologic effects that may relate to the disease entities, both colonic and systemic.

Table 2. Potential physiological effects of inulin<sup>a</sup>

Local	Systemic
↑ Fecal bulk	↓ Cholesterol
↑ Bacteria	↓ TG (↓ Insulin; ↓ Glucose)
Selective ↑ bacteria	↓ $\text{NH}_3$
↑ SCFA production	↓ Urea
Selective ↑ in SCFA	↑ B vitamins
↑ Mineral absorption	↑ Immune function
↑ B vitamin synthesis	(↑ Glutamine ?)

<sup>a</sup> Abbreviations: SCFA, short-chain fatty acids; TG, triglyceride (Adapted from Jenkins, et al. 1999).

## 3. Colonic Events

Possibly the most widely accepted effect of inulin is the changes in relative proportions of a variety of microorganisms, especially an increase in both the numbers and proportion of fecal Bifidobacteria. The physiological effects of bifidobacteria have only recently been studied (Tamime, 1998; Buddington, 2001). The effects of these anaerobic microbes are beyond the competitive exclusion of pathogenic bacteria. The conversion of inulin by bifidobacteria into

short chain carboxylic acids and lactic promotes events in the colon including gut health and productivity (Buddington, 2001). Furthermore, the presence of these microbes in the large intestine enhances fecal nitrogen excretion and reduces the excretion of renal nitrogen (Younes et al. 1995). This occurs because these fermentable carbohydrates serve as energy source for the intestinal bacteria, which, during growth, also require a source of nitrogen for protein synthesis. The inulin-type fructans because of their osmotic effect in the small intestine accelerates the transfer of urea into the distal ileum and the large intestine, where a highly ureolytic microflora may proliferate. Younes et al. (1995) concluded that when inulin intake is high, the amount of ammonia required to sustain maximal bacterial growth may become insufficient, and blood urea is then required as a ready source for bacterial protein synthesis in the cecum. Besides its effect in the gastrointestinal tract and its possible role in modulating lipogenesis, propionate, an important end product of bacterial fermentation of inulin-type fructans, also inhibits ureagenesis in the liver in the presence of ammonia and amino acids.

#### FRUCTANS IN SWINE DIETS

Most of the earlier research on the feeding of fructans to pigs started in Japan with the addition of Neosugar to the diet. Hidaka et al. (1986) reported increases in body weight and feed efficiency, and decreases in incidence of diarrhea and levels of putrefactive compounds in the feces. These results suggested beneficial effects of Neosugar fructans on growth and health of swine. In addition, intestinal Streptococci and Lactobacilli increased while Clostridia decreased. In another study, Fukuyasu and Oshida (1986) reported that inclusion of Neosugar as low as 0.25 and 0.5% of the diet improved weight gains by 35% and 73% and reduced diarrhea in field trials of 50 pigs as reported by Farnworth (1993). In addition, the levels of several putrefactive compounds in the feces of the inulin fed groups decreased with time. The decrease in the amounts of fecal putrefactive compounds is consistent with the concept that the population of intestinal microorganisms had changed due to the feeding of inulin. Recently, inulin has been classified as a prebiotic. A prebiotic is a "non-digestible feed ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health (Gibson and Roberfroid, 1995). Improving pig health is supposed to increase pig performance. However, Houdijk et al. (1998) indicated that inclusion of inulin in pig diets does not always improve growth performance. In experiment, Houdijk et al. (1998) exchanged cellulose for inulin in the diets of young pigs and reported a temporary lowered feed intake and apparent daily weight gain. However, the inclusion of inulin in pig diets did not affect mean growth performance during six weeks. Recent studies (Mroz et al. 1993; Bakker, 1996; Canh et al. 1997) with complex carbohydrates have shown that  $\beta$ -glucans and nonstarch polysaccharides can influence endogenous nitrogen excretion at the terminal ileum and microbial fermentation in the large colon, resulting in increased bacterial protein production and altered volatile fatty acid production.

A study was designed to determine the efficacy of inulin in water, feed or both for early weaned pigs. One hundred and eighty early weaned (17 – d old) pigs were allotted, based on body weight and sex, into five groups. The dietary treatments were as follows:

1. Basal diet as negative control
2. Basal diet supplemented with inulin in water for 14 days
3. Basal diet supplemented with inulin in water and feed for 14 days
4. Basal diet supplemented with inulin in feed
5. Basal diet supplemented with antibiotics.

Pigs were housed in 30 pens (1.5m × 1.8m) with a Tenderfoot floor. There were 6 pigs in each pen, Temperature was maintained between 26-22°C in thermostatically controlled room. Each pen had a nipple water fountain and a self-feeder. Animals had free access to feed and water always.

The duration of the trial was 4 weeks and was divided into 3 phases. Week 1 was phase 1, week 2 was phase 2 and week 3 to 4 was phase 3. There were 3 types of diets for each phase (Table 3). Diet 1 was a basal diet and fed to pigs in treatment 1 and treatment 2, diet 2 contained 0.5%, 0.2% and 0.1% inulin replacing part of corn meal for phase 1, 2 and 3, respectively, and was fed to pigs in treatment 3 and treatment 4. Diet 3 contained 0.25% antibiotics replacing part of corn meal for all 3 phases and was fed to pigs in treatment 5. All diets were formulated on ideal protein basis and all nutrients in the diets were supplied to meet or exceed current requirements for early weaned pigs (NRC, 1998).

Pigs were weighed and feed intake was determined for each phase. The change in body weight, feed intake per pen and the feed conversion ratio were determined. The incidence of diarrhea in pigs, if it occurred, was noted daily.

The GLM procedure of SAS was used to determine differences between treatment means. Values were considered significantly different at the  $P < 0.05$  level.

**Table 3. Composition of diets (%)**

Ingredients	Phase 1			Phase 2			Phase 3		
	Diet 1	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3
Corn Meal	43.41	42.91	43.16	53.61	53.41	53.36	60.99	60.89	60.74
Soybean Meal	28.33	28.33	28.33	27.00	27.00	27.00	28.4	28.4	28.4
Dried Whey	15.00	15.00	15.00	10.00	10.00	10.00	5.0	5.0	5.0
Fish Meal	6.00	6.00	6.00	4.00	4.00	4.00	3.0	3.0	3.0
Lime Stone	0.61	0.61	0.61	0.83	0.83	0.83	0.68	0.68	0.68
Dical P	0.63	0.63	0.63	0.55	0.55	0.55	0.66	0.66	0.66
Salt	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Vit-Min Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Choline	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Antibiotic			0.25			0.25			0.25
Inulin		0.50			0.20			0.10	
Spray Dried PP	5.00	5.00	5.00	2.74	2.74	2.74			
Tallow	0.25	0.25	0.25	0.5	0.5	0.5	0.5	0.5	0.5

**Table 4. Calculated nutrient content in experimental diets**

Nutrients	Phase 1 diets	Phase 2 Diets	Phase 3 Diets
ME, kcal/kg	3303	3327	3346
Crude Protein, %	25.5	22.1	20.0
Lysine, %	1.68	1.37	1.15
Met, %	0.42	0.36	0.34
Met + Cys, %	0.90	0.77	0.68
Threonine, %	1.12	0.93	0.78
Tryptophan, %	0.33	0.28	0.24
Calcium, %	0.90	0.81	0.70
Available P, %	0.55	0.42	0.34
Total P, %	0.79	0.66	0.60

There was no diarrhea detected during the experiment. The results of the effects of inulin and antibiotics addition to the basal diet on pigs' feed intake (FI), average daily gain (ADG) and feed: gain (FCR) are shown in Table 5. Feed intake and feed: gain was not significantly affected by the treatments. Except for phase 3, there was no significant difference among the treatment groups in average daily gain. Subsequent contrast indicated that the difference among treatment groups in phase 3 was due to the higher ADG for antibiotic group in comparison with other groups. However, when inulin was provided in water, feed or both, there was a trend for inulin supplementation to improve ADG and FCR comparing to negative control group during the 3 phases. (Figure 1 to 3)

In conclusion, inulin supplement in water or feed tends to improve ADG and FCR in piglets during the first 4 postweaning weeks.

**Table 5. Effect of inulin and antibiotics on pigs' feed intake, growth rate and feed efficiency from phase 1 to phase 3.**

Phase 1							
Treatments	No. of		initial WT(kg)	Final WT(kg)	ADFI(g/d)	ADG(g/d)	Feed/gain
	p						
-inulin-AB	5		6.33 ± 0.27	7.68 ± 0.46	213.8 ± 12.57	192.7 ± 13.81	1.14 ± 0.042
+inulin water	7		6.17 ± 0.36	7.63 ± 0.50	216.7 ± 10.62	208.5 ± 11.67	1.04 ± 0.036
+inulin water & feed	7		6.35 ± 0.17	7.78 ± 0.30	213.1 ± 10.62	203.9 ± 11.67	1.04 ± 0.036
+inulin feed	5		6.40 ± 0.25	7.82 ± 0.30	212.4 ± 12.57	189.8 ± 13.81	1.13 ± 0.042
AB	6		6.53 ± 0.21	7.91 ± 0.30	205.4 ± 11.48	196.2 ± 12.61	1.05 ± 0.039

Phase 2							
Treatments	No. of		initial WT(kg)	Final WT(kg)	ADFI(g/d)	ADG(g/d)	Feed/gain
	pens						
-inulin-AB	5		7.68 ± 0.46	10.03 ± 0.78	480.5 ± 19.06	335.7 ± 16.00	1.44 ± 0.051
+inulin water	7		7.63 ± 0.50	10.12 ± 0.71	499.0 ± 16.11	355.1 ± 13.53	1.41 ± 0.043
+inulin water & feed	7		7.78 ± 0.30	10.11 ± 0.32	464.4 ± 16.11	355.4 ± 13.53	1.31 ± 0.043
+inulin feed	5		7.82 ± 0.30	10.45 ± 0.48	482.7 ± 19.06	374.1 ± 16.00	1.29 ± 0.051
AB	6		7.91 ± 0.30	10.51 ± 0.41	524.0 ± 17.40	371.0 ± 14.61	1.42 ± 0.047

Phase 3							
Treatments	No. of		initial WT(kg)	Final WT(kg)	ADFI(g/d)	ADG(g/d) *	Feed/gain
	pens						
-inulin-AB	5		10.03 ± 0.78	15.64 ± 1.38	706.0 ± 26.59	399.9 ± 18.44	1.78 ± 0.072
+inulin water	7		10.12 ± 0.71	16.02 ± 0.59	712.0 ± 22.47	422.4 ± 15.59	1.69 ± 0.061
+inulin water & feed	7		10.11 ± 0.32	15.19 ± 1.39	685.3 ± 22.47	402.7 ± 15.59	1.71 ± 0.061
+inulin feed	5		10.45 ± 0.48	16.40 ± 0.72	703.2 ± 26.59	425.5 ± 18.44	1.66 ± 0.072
AB	6		10.51 ± 0.41	17.12 ± 0.90	731.9 ± 24.27	476.5 ± 16.84	1.54 ± 0.065

\*there is significant difference among treatments (P<0.05)

Overall							
Treatments	No. of		initial WT(kg)	Final WT(kg)	ADFI(g/d)	ADG(g/d)	Feed/gain
	pens						
-inulin-AB	5		6.33 ± 0.27	15.64 ± 1.38	526.6 ± 15.50	332.1 ± 12.53	1.59 ± 0.044
+inulin water	7		6.17 ± 0.36	16.02 ± 0.59	534.9 ± 13.10	352.1 ± 10.59	1.52 ± 0.037
+inulin water & feed	7		6.05 ± 0.17	15.19 ± 1.39	512.0 ± 13.10	341.2 ± 10.59	1.50 ± 0.037
+inulin feed	5		6.40 ± 0.25	16.40 ± 0.72	525.4 ± 15.50	353.8 ± 12.53	1.49 ± 0.044
AB	6		6.53 ± 0.21	17.12 ± 0.90	548.3 ± 14.15	380.0 ± 11.44	1.45 ± 0.040

(He et al. 2002)

## INULIN AND ENVIRONMENTAL IMPACT OF SWINE MANURE

The effects of inulin in the diet of growing pigs were evaluated in a digestibility study including nitrogen and phosphorus balance. Table 6 gives the composition of the experimental diets, which were formulated to meet or exceed requirements for growing pigs (NRC, 1998). Twelve pigs (GAP Genetics) weighing  $34.9 \pm 1.53$  lb were raised individually in metabolism crates for 12 days, including 7 days acclimation and 5 days sample collection period. Three dietary treatments were randomly assigned to each animal with 4 animals per treatment. Total of 2 kg feed was fed in three allotments each day per pig.

During sample collection days, a plastic bag was attached to each pig with velcro and medical glue. Total feces were collected in the plastic bags, pooled by animal and approximately 10% of the collection was sampled and stored at  $-20^{\circ}\text{C}$  for future analysis.

Table 6. Experimental diet composition and nutrient level

	Control	Control+0.1 % inulin	Control + 0.25% AB
Corn Meal	65.99	65.89	65.74
Soybean Meal	28.4	28.4	28.4
Fish Meal	3.0	3.0	3.0
Lime Stone	0.68	0.68	0.68
Dicalcium Phosphate	0.66	0.66	0.66
Salt	0.37	0.37	0.37
Vitamin-Mineral Premix <sup>1</sup>	0.25	0.25	0.25
Choline	0.15	0.15	0.15
Antibiotic	-	-	0.25
Inulin	-	0.10	-
Tallow	0.5	0.5	0.5
Determined nutrients level			
ME, Kcal/kg	3357.4	3354.0	3348.8
Crude protein, %	19.8	19.8	19.8
Lysine, %	1.12	1.12	1.12
Ca, %	0.66	0.66	0.66
Total P, %	0.58	0.58	0.58

There were no effect on daily feed intake, daily nitrogen intake due to dietary treatments (Table 7). However, 0.5% inulin or 0.25% AB in feed tend to result in less nitrogen excretion per unit of feed intake ( $p=0.09$ ), and daily nitrogen retention for pigs fed inulin or AB supplemented feed tend to be higher than control diet ( $p=0.05$ ). There were no effects of dietary supplementation of inulin or AB on phosphorous intake, excretion nor retention.

Table 7. Effect of inulin and AB supplementation on nitrogen and phosphorus balance

Parameters	Control	Control+0.5%inulin	Control+AB	s.e.	P value
Feed intake (gram/day)	2077.4	2152.0	2047.0	55.19	0.42
N intake (gram/day)	66.6	69.0	65.6	1.77	0.42
N excretion/intake, %	49.6	46.4	44.3	1.52	0.09
N retention, g/day	33.6	36.9	36.5	0.90	0.05
Fecal N: urine N	3.2	2.7	3.0	0.30	0.52
Apparent fecal N digestibility, %	87.8	87.2	89.0	1.08	0.50
P intake (gram/day)	11.5	11.9	11.3	0.30	0.42
P excretion/intake, %	42.6	46.3	37.1	6.39	0.61
P retention, gram/day	6.6	6.3	7.1	0.69	0.70

From this experiment, it was concluded that inclusion of 0.5% inulin to replace antibiotics for growing pigs did not affect feed intake but improved nitrogen retention and had no impact on phosphorus excretion.

## SUMMARY

Dietary carbohydrates have a wide variety of physiological and nutritional properties that demonstrates an array of potential health and possibly environmental benefits. Changes in the composition of the colonic microbiota, and the impact of such changes appear to be a promising area for further research. Gastro-intestinal infections are common in young pigs. This might perhaps be due to the changes in intestinal microflora that occur during the transition from milk to solid food. Young pigs could benefit from the addition of inulin in the diet, which will result in increase in bifidobacteria in the gastrointestinal tract. By means of competitive mechanisms, bifidobacteria hamper the growth of potentially pathogenic bacteria by: production of short carboxylic acids and lactic acid, reduction of colonic pH, production of antibiotic substances and reduction of the formation of putrefactive factors and toxins like ammonia, phenols, indole and secondary bile acids. The pigs' nutritional needs and efficacy of dietary fibers should be evaluated in terms of finding a substitute for anti-microbial growth promoters.

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# THE RELATIONSHIP BETWEEN DISTILLER'S DRIED GRAINS WITH SOLUBLES (DDGS) AND ILEITIS IN SWINE

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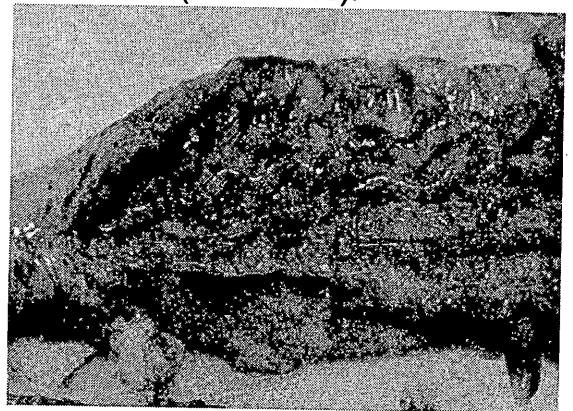
## WHAT IS ILEITIS?

Ileitis, also known as porcine proliferative enteropathy, is an enteric disease of the lower small intestine and occasionally large intestine that can decrease feed intake, reduce growth performance, and increase mortality in swine. The disease is caused by a gram negative microaerophil bacteria called *Lawsonia intracellularis*, an intracellular parasite of the enterocytes of the intestine (Dufresne, 1999). *Lawsonia intracellularis* infects immature epithelial cells located in the crypts of the intestine, keeping them from maturing and causing them to multiply without leaving the intestine. This results in cellular proliferation and thickening of the infected intestine (primarily ileum and ileocaecal junction), and can result in necrosis, ulceration, and hemorrhaging of the epithelial surface (see Figure 1). Tests using the National Animal Health Monitoring System (NAHMS) serum bank indicate that *Lawsonia intracellularis* is present in 96% of all U.S. swine herds (Bane et al., 1997). This disease has been estimated to cost the U.S. pork industry \$20 million annually, based on calculations utilizing NAHMS data, and as much as \$8.50 per pig in an infected herd.

Figure 1. Normal ileum and ileum with gross thickening and necrosis indicating porcine intestinal adenomatosis (PIA - ileitis).



Healthy Ileum



PIA lesion in ileum

Ileitis can affect pigs at any time after weaning, but is most commonly found in 40 to 100 lb. growing pigs, bred gilts, sows, and boars and occasionally in finishing pigs (Glock et al., 1994). Generally 1 to 10% of animals are affected in a herd, although this prevalence may rise as high as 50% in young growing pigs.

Animals are infected by oral contact with the bacteria shed in feces from other infected animals. Lesions of the intestinal wall begin to form 7 to 10 days after infection, but reach their maximum approximately 21 days after infection. The disease expresses itself clinically in one of two forms: porcine intestinal adenomatosis (PIA) and porcine hemorrhagic enteropathy (PHE) (Dufresne, 1999). PIA is a chronic condition generally seen in pigs between 6 and 20 weeks of age, and results in decreased feed intake and a lethargic or unthrifty appearance. Growth performance and feed conversion are negatively affected, and diarrhea is often observed. Expression of the disease appears to be triggered when environmental stressors are applied to animals.

Ileitis has been a difficult disease for the swine industry to control. Strict biosecurity measures are necessary to prevent the spread of the disease from one site to the next. In fact, many species of animals have been identified as potential carriers of the disease, including rabbits, hamsters, deer, horses, ostriches (Cooper et al., 1997). Early weaning and use of multiple sites has not been successful in controlling the disease, and this may be due to carrier gilts infecting their piglets very early in life (Dufresne, 1999). Antibiotics and/or antimicrobials, including tetracycline, tylosin, lincosamides, tiamulin, and carbadox, have been used effectively against acute breaks of *Lawsonia intracellularis*, but have been less successful in prevention of disease. Sub-therapeutic levels of these antibiotics often fail to prevent the disease, while therapeutic levels of feed-grade antibiotics are very expensive to maintain in the diet. In addition, public safety concerns over potential residue violations in meat and the risk of antibiotic-resistance in human strains of pathogenic organisms precludes continued use of these drugs if not necessary.

### DOES DISTILLER'S DRIED GRAINS WITH SOLUBLES PLAY A ROLE IN CONTROLLING ILEITIS?

There have been several field reports suggesting that including distiller's dried grains with solubles (DDGS) in grow-finish diets in commercial herds with a history of previous ileitis problems may improve the pig's ability to resist or recover from ileitis outbreaks, and thus may reduce dependence on antibiotics to combat this disease. At dietary inclusion levels of 5 – 15% DDGS in grow-finish diets, some producers have reported that they have reduced or completely eliminated the use of antibiotics without any negative effects on growth performance and mortality.

DDGS is a co-product of the ethanol industry that is suitable as a livestock feed, and contains approximately 10% crude fiber. Historically, the majority of DDGS has been utilized in ruminant diets, but results from several research studies conducted by our group at the University of Minnesota have shown that DDGS originating from "new-generation" ethanol plants located in the upper Midwest is an excellent ingredient for non-ruminant diets as well. Although "new generation"

DDGS contains a significant amount of crude fiber (8 – 10%), it also contains 10 – 12% fat (Whitney et al., 1999), resulting in considerably higher energy values (approximately 3950 kcal/kg DE or 3800 kcal/kg ME, respectively, on a dry matter basis), compared to 3440 kcal/kg DE or 3030 kcal/kg ME reported in NRC (1998). DDGS is also an excellent source of available phosphorus (0.80%) (Whitney et al., 2001), and contributes significant levels of apparent ileal digestible lysine, methionine, threonine, and tryptophan (0.44, 0.32, 0.62, and 0.15%, respectively), but, like corn, the amino acid profile is poor, relative to the pig's requirements (Whitney et al., 2000). Thus, adding DDGS to swine diets increases nitrogen and decreases phosphorus excretion (Spiehs et al., 1999), and can play an important role in manure nutrient management.

Laboratory analysis conducted by our group suggests that the fiber composition is primarily insoluble (42.2%) versus soluble (0.7%) in nature (Shurson et al., 2000). According to Hampson (1999), feeding diets that are low in soluble non-starch polysaccharides can reduce the proliferation of pathogenic organisms in the gastrointestinal tract. Providing less soluble, and more insoluble fiber in the diet results in less available substrate for organisms in the small intestine, and particularly the large intestine, and thus may reduce pathogen load. Smith and Halls (1968) suggested that fiber influences the secretory function of the epithelium, and this alteration may impair bacterial adhesion. Fiber also has a "cleansing" effect in the gut as a result of reducing the viscosity of digesta (Lawrence, 1972). Additionally, a considerable amount of "spent" yeast, remaining from the ethanol fermentation process, is present in DDGS. Yeast cells are an excellent source of mannan-oligosaccharides (MOS). MOS have been shown to serve as alternative attachment sites for certain bacteria, thereby blocking attachment to the intestinal wall, and in the case of pathogens, eliciting an immune response (Van der Beke, 1997).

It is quite possible that the fiber or yeast cells remaining in DDGS have the ability to promote gastrointestinal health by any or all of the mechanisms described above. In order to evaluate these potential DDGS benefits, our group has developed a series of studies using an ileitis disease challenge model for evaluating nutritional effects on gut health. By using this model, we have attempted to determine if field observations could be simulated and measured in a controlled research setting. Two disease challenge studies have been completed to date, and have focused on evaluating the effects of DDGS and/or antibiotic/antimicrobial regimen on control of an ileitis infection. Future studies are being planned to evaluate the effects of other fiber sources and feed additives.

## RESEARCH STUDIES TO EVALUATE THE EFFECT OF DDGS ON CONTROLLING AN ILEITIS INFECTION

### Experiment 1: Effect of DDGS inclusion on ability of the young growing pig to resist an ileitis challenge

**Objectives:** (1) Develop a disease challenge model that can be utilized to evaluate nutritional effects on pig resistance to an ileitis challenge, (2) determine if dietary inclusion of DDGS affects the incidence or severity of ileitis in growing pigs, and (3) determine which dietary level of DDGS (10 or 20%) elicits the greatest response in the pig during an ileitis challenge.

**Procedures:** 80 crossbred pigs (40 gilts, 40 barrows) were weaned at 17 days of age, and were transported to the CVM-RAR isolation barns located on the University of Minnesota St. Paul campus, and randomly allotted (blocked by sex and weight) to one of four treatments. Pigs were housed in separate rooms (10 pigs/room, 2 rooms/treatment). All pigs were fed a commercial Phase 1 pelleted diet during the first 4 days of the trial, and were subsequently placed on their respective experimental diets for the remainder of the study. Experimental diets were formulated to contain equivalent energy (3390 kcal/kg ME), calcium (0.80%), total phosphorus (0.70%), and apparent ileal digestible lysine (1.15%).

<u>Treatment:</u>	<u>Diet:</u>	<u>Lawsonia Challenge:</u>
(1) Negative Control (NC)	Corn-soybean meal	No
(2) Positive Control (PC)	Corn-soybean meal	Yes
(3) 10% DDGS (D10)	10% DDGS	Yes
(4) 20% DDGS (D20)	20% DDGS	Yes

Four weeks after experimental diets were initiated, pigs were provided 60 ml of either saline (NC) or an inoculation of *Lawsonia intracellularis* (PC, D10, and D20 treatments) via stomach tube. The inoculate was prepared as a mucosal homogenate collected from the small intestines of pigs previously infected with *Lawsonia intracellularis* and exhibiting lesions consistent with ileitis. Care was taken to avoid cross-contaminating pigs from different rooms. Growth and feed intake data were collected for the pre- and post-inoculation periods. Pigs were observed for gauntness and lethargy, and fecal scores indicating degree of firmness or looseness were taken. Fecal samples were collected on day 14 and 20 post-inoculation, and sent to the University of Minnesota Diagnostic Laboratory for PCR evaluation of *L. intracellularis* shedding. On day 20 or 21 post-inoculation, all pigs were euthanized and necropsies were performed to visually evaluate lesions, and to collect ileal tissue samples for immunohistochemistry (IHC) testing of *L. intracellularis* presence and proliferation.

Growth performance data were analyzed by room using analysis of variance, (two replications per treatment). All other data were analyzed utilizing the individual pig as the experimental unit, giving 20 replications per treatment. Least squares means were used to compare the negative and positive controls, and to evaluate the effects of infected pigs on response criteria. Analysis of variance was conducted to compare response criteria among the disease challenge treatments (PC, D10, and D20). In addition, least squares means comparisons were conducted between challenged treatments to identify differences due to dietary DDGS inclusion level.

**Results:** All pigs remained on test for the duration of the experiment. Body weights, growth performance, feed intake, and feed efficiency results are shown in Table 1. Average initial pig weight was 5.7 kg at the beginning of the trial. During the pre-challenge period, feed intake and feed efficiency were similar across all treatments, although pigs fed the 10% DDGS diet tended to grow slightly faster than pigs fed the 20% DDGS diet. Infecting pigs with *L. intracellularis* greatly reduced feed intake, growth, and feed efficiency by 25, 55, and 40%, respectively, during the 3-week post-challenge period compared to uninfected pigs ( $P < 0.01$ ). In addition, looser fecal consistency was observed (data not shown) from day 5 to day 20 post-challenge in challenged pigs compared to unchallenged pigs ( $P < 0.10$ ). Dietary treatment (0, 10, or 20% DDGS) did not affect growth, feed intake, or feed conversion responses post-challenge, however, and resulted in similar end body weights.

**Table 1. Effect of dietary distiller's dried grains with solubles inclusion and ileitis challenge on growth performance, feed intake, and feed conversion efficiency.**

	NC	Challenged Trts			NC vs PC Pr>F	Challenged Trts	
		PC	D10	D20		Mean	Pr>F
# of pens	2	2	2	2		6	
<b>Body weight</b>							
Initial, kg	5.7	5.7	5.7	5.7	0.99	5.7	0.99
Challenge, kg	16.7	17.5	17.8	16.9	0.26	17.4	0.51
Final, kg	29.9	24.5	23.7	22.6	0.01	23.6	0.36
<b>Pre-challenge (day 0 - 32)</b>							
ADG, g	354	379 <sup>a,b</sup>	389 <sup>a</sup>	360 <sup>b</sup>	0.14	374	0.15
ADFI, g	567	595	593	589	0.16	592	0.97
G/F	0.62	0.64	0.66	0.61	0.60	0.64	0.43
<b>Post-challenge (day 32 - 53)</b>							
ADG, g	600	311	259	245	0.01	272	0.67
ADFI, g	1363	990	1012	1067	0.01	1023	0.70
G/F	0.44	0.31	0.26	0.23	0.01	0.27	0.43

<sup>a,b</sup> Different superscripts indicate difference between means within challenged treatments ( $P < 0.10$ ).

Necropsy results are presented in Table 2. No lesions were observed for the negative control group. Overall, 63% percent of pigs that were challenged exhibited lesions consistent with ileitis. No dietary effects on prevalence were observed, although pigs fed the 10% DDGS diet had more area (length) of lesions recorded compared to pigs fed the control 0% DDGS diet, with pigs fed the 20% DDGS diet intermediate. These results are consistent with jejunum lesion data, although pigs fed either DDGS level tended to have more severe lesions (higher score), indicating a higher level of infection. However, no dietary differences were noted in lesion length, severity, or prevalence in the ileum.

**Table 2. Effect of dietary distiller's dried grains with solubles inclusion and ileitis challenge on lesion location, length, severity, and prevalence.**

	NC	Challenged Trts			NC vs PC Pr>F	Challenged Trts	
		PC	D10	D20		Mean	Pr>F
<b># of pigs</b>	20	20	20	20		60	
<b>Jejunum</b>							
Length, cm	0.0	15 <sup>a</sup>	54.4 <sup>b</sup>	31.9 <sup>a,b</sup>	0.02	33.8	0.16
Score (0-4)	0.0	0.4 <sup>a</sup>	1.1 <sup>b</sup>	1.2 <sup>b</sup>	0.01	0.9	0.08
Prevalence, %	0.0	20.0 <sup>a</sup>	50.0 <sup>b</sup>	45.0 <sup>b</sup>	0.01	38.3	0.12
<b>Ileum</b>							
Length, cm	0.0	7.5	11.8	11.1	0.01	10.1	0.39
Score (0-4)	0.0	0.9	1.5	1.5	0.01	1.3	0.22
Prevalence, %	0.0	50.0	65.0	60.0	0.01	58.3	0.63
<b>Cecum</b>							
Length, cm	0.0	0.0 <sup>a</sup>	1.5 <sup>b</sup>	0.15 <sup>a</sup>	0.25	0.5	0.05
Score (0-4)	0.0	0.0 <sup>a</sup>	0.5 <sup>b</sup>	0.05 <sup>a</sup>	0.21	0.2	0.03
Prevalence, %	0.0	0.0 <sup>a</sup>	20.0 <sup>b</sup>	5.0 <sup>a</sup>	0.19	8.3	0.06
<b>Colon</b>							
Length, cm	0.0	1.0	6.2	0.6	0.30	2.6	0.20
Score (0-4)	0.0	0.3 <sup>a</sup>	0.7 <sup>b</sup>	0.2 <sup>a</sup>	0.06	0.4	0.10
Prevalence, %	0.0	20.0	25.0	10.0	0.04	18.3	0.47
<b>Total</b>							
Length, cm	0.0	23.4 <sup>a</sup>	73.8 <sup>b</sup>	43.7 <sup>a,b</sup>	0.01	47.0	0.09
Prevalence, %	0.0	55.0	70.0	65.0	0.01	63.3	0.62

<sup>a,b</sup> Different superscripts indicate difference between means within challenged treatments ( $P < 0.10$ ).

Laboratory results are presented in Table 3. The PCR technique for determining *L. intracellularis* presence in feces is currently the most precise technique for testing ileitis in the live pig. Four negative control (NC) pigs on day 14 post-challenge, and 8 NC pigs on day 20 post-challenge tested positive, indicating that some cross-contamination between rooms occurred after challenge. By day 20 post-challenge, 80 – 100% of the inoculated pigs tested positive for shedding *Lawsonia*. A slightly higher percentage of pigs fed the DDGS diets tested

positive (95 – 100%) compared to positive control pigs (80%). Immunohistochemistry (IHC) results, however, indicated no difference in concentration or percentage of pigs testing positive for *L. intracellularis*. IHC is currently the most sensitive and accurate method of evaluating presence of ileitis, but requires submission of intestinal tissue for laboratory analysis, and therefore, involves sacrificing pigs. IHC results indicated that 30% of the NC pigs were exposed and contacted ileitis, but that the disease was in an early stage of infection by the end of the study.

**Table 3. Effect of dietary distiller's dried grains with solubles inclusion and ileitis challenge on fecal PCR and ileum IHC results.**

Test	NC	Challenged Trts			NC vs PC	Challenged Trts	
		PC	D10	D20	Pr>F	Mean	Pr>F
<b>Fecal PCR</b>							
Day 0	0.0	0.0	0.0	0.0	*	0.0	*
Day 14	20.0	70.0 <sup>a</sup>	90.0 <sup>b</sup>	90.0 <sup>b</sup>	0.01	83.3	0.15
Day 20	40.0	80.0 <sup>a</sup>	95.0 <sup>b</sup>	100.0 <sup>b</sup>	0.01	91.7	0.06
<b>IHC</b>							
Score (0-4)	0.55	2.00	2.15	2.25	0.01	2.13	0.71
Prevalence, %	30.0	100.0	90.0	95.0	0.01	95.0	0.36

<sup>a,b</sup> Different superscripts indicate difference between means within challenged treatments ( $P < 0.10$ ).

The target dose of *L. intracellularis* for this study was  $1 \times 10^8$ . This target dosage was difficult to achieve because the inoculate is a mucosal homogenate that is harvested from infected tissues on the day of inoculating the pigs. Therefore, laboratory quantification of the actual concentration of *L. intracellularis* is not possible prior to the disease challenge. We later determined that the actual concentration of *L. intracellularis* used was  $2.6 \times 10^7$  per ml, or a dosage rate of  $1.56 \times 10^9$ . Since this was considerably higher than our original goal, and visual observations during post-challenge and necropsy indicated that animals were more severely infected than normally would be observed in the field, we believe that any possible nutritional benefits of feeding DDGS on controlling ileitis may have been masked by the extremely high dosage rate of *Lawsonia*. Therefore, we chose to modify the subsequent disease challenge study by lowering the dosage rate.

**Conclusion:** Results from this experiment suggest that dietary inclusion of DDGS had no effect on the pig's ability to resist an ileitis challenge. The inoculation dosage used in this study, however, was much higher than our original target, and may have masked any potential dietary effects that would otherwise have been observed.

Experiment 2: Effect of DDGS and/or antibiotic regimen on ability of the young growing pig to resist an ileitis challenge

Objectives: (1) Modify the disease challenge model to provide an infectious dose comparable to level of exposure in commercial finishing barns, (2) determine if dietary inclusion of DDGS can reduce the incidence or severity of ileitis in growing pigs, and (3) compare dietary DDGS inclusion to an antibiotic regimen currently used to treat ileitis.

Procedures: 100 crossbred pigs (50 gilts, 50 barrows) were weaned at 17 days of age, were transported to the CVM-RAR isolation barns located on the University of Minnesota St. Paul campus, and randomly allotted (blocked by sex and weight) to one of five treatments. Pigs were housed in separate rooms (10 pigs/room, 2 rooms/treatment). All pigs were fed a commercial Phase 1 pelleted diet during the first 4 days of the trial, and were subsequently placed on their respective diets for the remainder of the study. Experimental diets were formulated to be equivalent in energy (3390 kcal/kg ME), calcium (0.80%), total phosphorus (0.70%), and apparent ileal digestible lysine (1.15%). Pigs were fed either a corn-soybean meal diet or a corn-soybean meal-10% DDGS diet, with or without antibiotics. The antibiotic regimen consisted of continuous BMD<sup>®</sup> inclusion (30 g/ton of mixed feed) along with pulsing of Aureomycin<sup>®</sup> (500 g/ton of mixed feed) from day 3 pre-challenge to day 11 post-challenge.

<u>Treatment:</u>	<u>Diet:</u>	<u>Antibiotic:</u>
(1) Negative Control (NC)	Corn-soybean meal	No
(2) Positive Control (PC)*	Corn-soybean meal	No
(3) DDGS (D)*	10% DDGS	No
(4) Control & Antibiotic (PC + A)*	Corn-soybean meal	Yes
(5) DDGS & Antibiotic (D + A)*	10% DDGS	Yes

\* indicates pigs were inoculated with a mucosal homogenate 4 weeks after initiation of dietary treatments

Data involving pigs on the disease challenge treatments were analyzed as a 2 x 2 factorial (with DDGS level (0 or 10%) and antibiotic regimen as the factors). All animal management procedures and data collection were conducted similar to those described for Experiment 1, except that the dosage rate was reduced when infecting pigs in the disease challenge treatment groups.

Results: Two pigs were removed from the experiment prior to completion due to health reasons unrelated to the ileitis challenge. Body weights, growth performance, feed intake, and feed efficiency results are shown in Table 4. Average initial pig weight was 6.7 kg at the beginning of the trial. During the pre-challenge period, growth, feed intake and feed efficiency were similar across all treatments.

**Table 4. Effect of dietary distiller's dried grains with solubles and or BMD/CTC inclusion under an ileitis challenge on growth performance, feed intake, and feed conversion efficiency.**

	NC	Challenged Trts				NC vs PC Pr>F	Challenge		P-value		
		PC	D	PC+A	D+A		Mean	Pr>F	D	A	DxA
# of pens	2	2	2	2	2		8		4	4	2
<b>Body weight</b>											
Initial, kg	6.6	6.9	6.8	6.6	6.7	0.11	6.7	0.45	0.92	0.18	0.46
Challenge, kg	19.5	20.8	19.2	19.9	20.0	0.26	20.0	0.61	0.40	0.98	0.35
Final, kg	36.3	34.9	30.6	33.4	35.1	0.65	33.5	0.47	0.57	0.50	0.22
<b>Pre-challenge (day 0 - 32)</b>											
ADG, g	404	432	386	417	416	0.41	412.8	0.65	0.40	0.79	0.41
ADFI, g	695	645	726	731	692	0.35	698.6	0.23	0.47	0.39	0.09
G:F	0.584	0.670	0.533	0.573	0.603	0.27	0.595	0.38	0.34	0.80	0.17
<b>Post-challenge (day 32 - 53)</b>											
ADG, g	799	672	542	642	720	0.29	644.2	0.49	0.75	0.39	0.25
ADFI, g	1262	1148	1046	1167	1276	0.52	1159.3	0.67	0.98	0.38	0.45
G:F	0.632	0.589	0.517	0.550	0.578	0.65	0.559	0.89	0.77	0.88	0.52

Infecting pigs with *L. intracellularis* appeared to reduce feed intake, growth, and feed efficiency during the 3-week post-challenge period, but these effects were not significant. Providing the combination of DDGS and antibiotic regimen together for challenged pigs resulted in similar feed intakes to negative control pigs, and appeared to partially make up for the drop in growth performance observed in other challenged pigs, but these mean differences were also statistically insignificant. DDGS or antibiotic regimen alone did not appear to affect performance. It should be noted that only 2 replications per treatment were used in the analysis of growth performance (room was the experimental unit), and that more replication is required to determine if numerical differences in treatment means observed are in fact dietary responses that could be expected on a consistent basis under similar conditions. This experiment, however, was designed to use lesion (necropsy) data and testing results as the primary response criteria, and growth performance as a secondary response parameter.

Looser fecal consistency was observed (data not shown) from day 3 to day 20 post-challenge in challenged pigs compared to uninfected pigs, and pigs fed the combination of DDGS and antibiotic regimen tended to have improved stool scores during the final week of the study ( $P < 0.15$ ).

Necropsy results for Experiment 2 are presented in Table 5. Two pigs in the negative control (NC) group had lesions that were suspect for ileitis. Overall, 59% percent of pigs that were challenged exhibited lesions consistent with ileitis, which was similar to Experiment 1. Feeding the 10% DDGS diet appeared to

reduce lesion length, severity, and percentage of pigs exhibiting lesions in the ileum, and to a lesser degree in the colon, resulting in an overall decrease in percentage of pigs with lesions. There was also a numerical trend for pigs fed the 10% DDGS diet to have reduced lesion length. Continuous BMD<sup>®</sup> inclusion with pulsing of Aureomycin<sup>®</sup> reduced severity of lesions and percentage of pigs exhibiting lesions in the jejunum, and resulted in a numerical trend toward an overall reduction in lesion length.

**Table 5. Effect of dietary distiller's dried grains with solubles and or BMD/Aureomycin inclusion under an ileitis challenge on lesion location, length, severity, and prevalence.**

	NC	Challenged Trts				NC vs PC Pr>F	Challenge		P-value		
		PC	D	PC+A	D+A		Mean	Pr>F	D	A	DxA
# of pigs	19	19	20	20	20	38	79		40	40	20
<i>Jejunum</i>											
Length, cm	1.26	22.16	14.65	8.60	10.20	0.02	13.80	0.49	0.68	0.18	0.50
Score (0-4)	0.05	0.90	0.38	0.28	0.25	0.01	0.45	0.03	0.11	0.03	0.16
Prevalence, %	5.3	47.4	30.0	20.0	15.0	0.01	27.8	0.12	0.28	0.04	0.54
<i>Ileum</i>											
Length, cm	0.37	10.58	5.50	9.75	6.40	0.01	8.03	0.11	0.02	0.98	0.62
Score (0-4)	0.05	1.54	0.75	1.43	1.05	0.01	1.19	0.10	0.02	0.70	0.40
Prevalence, %	5.3	68.4	40.0	80.0	55.0	0.01	60.8	0.06	0.02	0.22	0.87
<i>Cecum</i>											
Length, cm	0.00	0.16	0.25	0.30	0.00	0.57	0.18	0.76	0.62	0.79	0.36
Score (0-4)	0.00	0.05	0.05	0.05	0.00	0.36	0.04	0.80	0.55	0.55	0.59
Prevalence, %	0.0	5.3	5.0	5.0	0.0	0.36	3.8	0.80	0.55	0.55	0.59
<i>Colon</i>											
Length, cm	0.00	2.11	0.30	1.20	0.50	0.01	1.01	0.08	0.02	0.51	0.30
Score (0-4)	0.00	0.47	0.10	0.20	0.15	0.01	0.23	0.15	0.09	0.37	0.19
Prevalence, %	0.00	31.6	5.0	20.0	10.0	0.01	16.5	0.12	0.03	0.70	0.32
<i>Total</i>											
Length, cm	1.63	35.05	20.40	19.45	11.35	0.01	21.39	0.19	0.14	0.11	0.67
Prevalence, %	10.5	68.4	40.0	80.0	50.0	0.01	59.0	0.04	0.01	0.32	0.94

Fecal PCR and ileum tissue IHC results are presented in Table 6. Challenging pigs with *Lawsonia* resulted in a 97.5% detection rate of *L. intracellularis* in ileal tissue, indicating that nearly all pigs were successfully infected with ileitis. Although the combination of DDGS and antibiotic regimen appeared to affect fecal shedding 14 days post-challenge, there were no dietary effects on shedding by 20 days post-challenge, and ileum IHC indicated no dietary effect on percentage of pigs testing positive for ileitis. IHC scores (indicating proportion of cells infected with *L. intracellularis*) did, however, indicate a positive effect of DDGS and antibiotic regimen on reducing concentration, or severity, of the infection.

**Table 6. Effect of dietary distiller's dried grains with solubles and or BMD/Aureomycin inclusion under an ileitis challenge on fecal PCR and ileal tissue IHC scores.**

	NC	Challenged Trts				NC vs PC Pr>F	Challenge		P-value		
		PC	D	PC+A	D+A		Mean	Pr>F	D	A	DxA
<i>IHC</i>											
Score (0-4)	0.00	2.58	1.95	2.00	1.90	0.01	2.10	0.05	0.05	0.10	0.16
Prevalence, %	0.0	100.0	95.0	100.0	95.0	0.01	97.5	0.59	0.17	1.00	1.00
<i>Fecal PCR</i>											
Day 14	0	63.2	25	25	40	0.01	40	0.04	0.28	0.28	0.02
Day 20	0	68.4	60	65	45	0.01	59.5	0.47	0.21	0.41	0.61

**Conclusion:** Results from this study suggest that including 10% DDGS in growing pig diets may provide some protection and aid the pig in coping with ileitis under a disease challenge situation. These results are consistent with field reports suggesting that DDGS inclusion results in reduced severity of clinical signs during an ileitis outbreak. The beneficial effects observed during this study were similar to the results observed for an approved antibiotic regimen (BMD<sup>®</sup> with 14-day Aureomycin<sup>®</sup> pulse). The BMD<sup>®</sup>/Aureomycin<sup>®</sup> regimen used in this study has been consistently shown in previous studies to aid in the treatment of ileitis, and results have been similar to observations from this trial. An additive effect of DDGS and BMD/Aureomycin was not statistically observed in this study, but variation in data collected and/or number of replications may have prevented detection of growth and/or lesion differences. The lower inoculation dosage rate used for this study (compared to Experiment 1) was quite successful in infecting most pigs, and appeared to be a more appropriate level of infection, allowing for detection of dietary effects on the pig's ability to resist an ileitis infection.

## FUTURE RESEARCH

Two studies are planned and scheduled for completion during the next year. One study is a collaborative effort involving our group at the University of Minnesota and researchers at South Dakota State University, in which animals will be raised in a more typical commercial grow-finish environment. Animals will be fed either a corn-soybean meal control diet or diets containing either DDGS or soy hulls. Some field reports have suggested that similar responses observed in Experiment 2 when feeding DDGS to pigs challenged with ileitis have also been observed when adding soy hulls to grow-finish diets. Animals will be inoculated similar to the procedures described for Experiment 2, but animals will not be sacrificed and growth and mortality data will be recorded over the entire grow-finish period. This study is scheduled to begin in August or September of 2002.

The next study being planned is also a disease challenge study that will be conducted using procedures similar to those described for Experiment 2. Dietary alternatives planned for testing include DDGS, soy hulls, and a polyclonal antibody feed additive product due to be marketed beginning in 2003. This study is scheduled to begin in October of 2002.

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