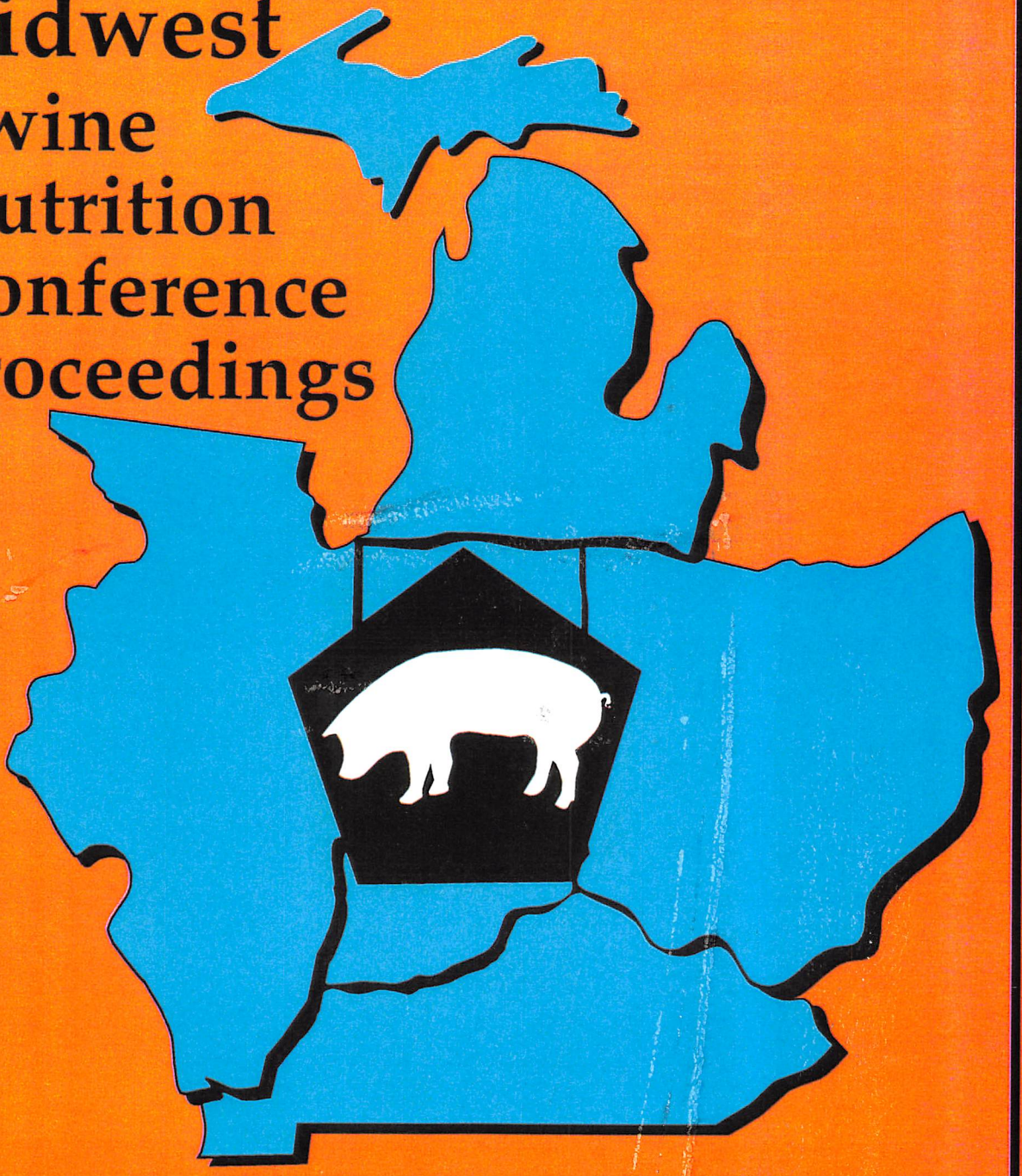
Midwest Swine Nutrition Conference Proceedings



Indianapolis, Indiana — September 8, 2005

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Appreciation is expressed to the Indiana Farm Bureau and their staff for hosting the Midwest Swine Nutrition Conference and providing the facilities for this activity.

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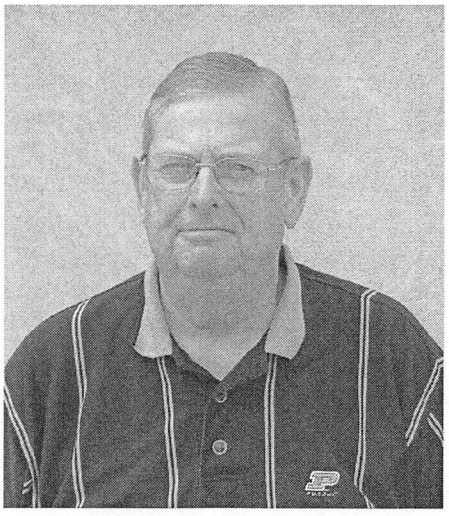
CONTENTS

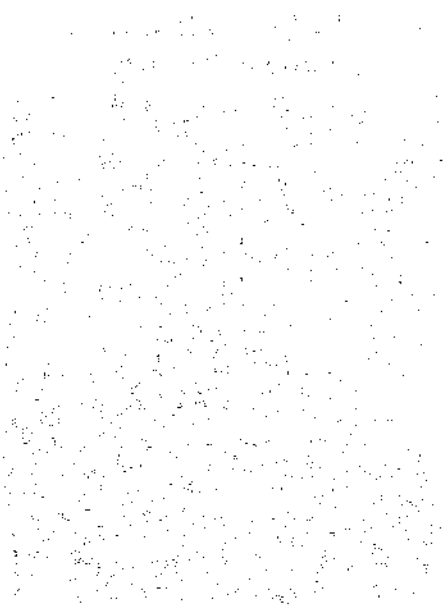
In Honor of The Retirement of

Dr. Tilford R. Cline
Purdue University



Dr. T.R. Cline or more commonly called "Tip" retired from Purdue University on August 31, 2005. Although making a career in both research and teaching Tip has primarily distinguished himself by spending the majority of his effort in teaching undergraduate and graduate students for over 40 years as a faculty member in the Animal Science Department at Purdue University. His excitement in teaching students continued throughout his career. He has thus received numerous recognitions for his teaching efforts. In 1976 he won the Animal Science Outstanding Teacher Award (Midwest section) and received the American Society Fellow Award in teaching in 1998. Dr. Cline was one of the first involved in studies evaluating high lysine corn and other mutants that resulted in improved corn genotypes used extensively today in many areas of the world. He was one of the first to advocate split sex feeding of pigs and was part of the team that investigated the all-in, all-out management-nutrition programs for grower finisher swine. Dr. Cline was born and raised on a livestock and grain farm in West Central Illinois (Virginia, Illinois) where he graduated from High School in 1956. He subsequently attended and graduated from the University of Illinois with a BS degree in 1960, a M.S. degree in 1962, and a PhD degree in 1965. He served in a post-doctoral position at Purdue University from 1965-1966, hired as an Assistant Professor in 1966, whereupon he rose rapidly to the rank of Professor by 1977. Upon his retirement, on September 1, 2005 he received Professor Emeritus status.

Dr. Cline was one of the co-founders of the Tri-State Nutrition Committee (IN, OH, MI), where a swine nutrition-management bulletin was published in 1998. The Midwest Swine Nutrition Conference was an outgrowth of that original committee and was started in 2001. This conference has continually grown and is presented annually and now includes the swine nutrition faculty from five states (KY, IL, IN, OH, MI) as committee members. Dr. Cline provided leadership in all aspects of this collaborative effort. He has also been deeply involved and served as Coordinator of the Pork Industry Handbook for a 10 year period. He has been an active member of the North Central Regional Swine Nutrition Committee (NCR-42). His friendly demure and "common sense" approach in the area of swine nutrition will be sorely missed.



Nutritional Concerns as Viewed by the Feed Industry

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Summary

The reduction of research funds and personnel is a concern to the feed industry as the quantity and quality of swine nutrition research will be impacted. Feed industry, and allied industry research may present some drawbacks. Practical swine nutrition research will suffer the most. Ingredient matrices need to be more reliable and representative of the current ingredient inputs. The advent of increased amino acid additions and the influx of new ethanol plants only emphasize the need for improved analytical procedures of ingredient inputs. The longevity and application of feed additives are important complements to swine nutrition. The concern is that scientific evidence prevails with respect to their continued use. The final concern of additional regulations is a fact of life for the industry. The hope is that science will prevail when more regulations are established.

Introduction

There are probably many concerns or uncertainties that the feed industry is encountering or about to encounter. Many may be classified as non-nutritional. Individual concerns may vary depending upon responsibilities, and length of tenure in the industry. The author's experience is primarily in the areas of formulation, product development and management, and technical support of a multi-level sales staff.

The concerns to be addressed are long term quality and quantity of nutritional research. The reliability of ingredient matrices is a major concern as they are necessary to solve nutrient related challenges. In addition, the future of feed additives, and additional government regulations, as each affects nutrition, is of concern to the feed industry.

Research

The funds available for research are being challenged. The feed industry has depended on university and experiment station trials for much of the basic and some of the practical answers to swine nutrition. As the number of researchers diminishes and moneys are more difficult to obtain, the quantity of research has to be reduced, especially of a practical nature. The value of quality unbiased, refereed data may become more difficult to obtain in the future.

Most of the feed industry participants support some form of company research. The problem is that company research may be mostly proprietary. Feed industry research is not always open to challenges, usually of a product development nature, and could contain a high degree of tunnel vision. In any case, the information may not help answer practical nutritional questions, may not be reported to anyone outside the research committee, and may not be adequately challenged.

If these previously stated suggestions are even partially true, several responses could and are taking place. First, the influx of allied industry and European data may be used to fill a void and to support various products and applications. Allied industry data may be biased by design. When some of these products, techniques, and procedures are applied practically, the responses may not be as claimed. The recent influx of European allied industry research lacks adequate experimental design, number of experimental units, and statistical results. It must also be stated that there are quality data from some of these sources.

There also seems to be some recent efforts to promote ideas and products through anecdotal evidence. This is certainly not a very scientific way to promote animal performance but it's cheaper than doing the basic research to determine efficacy.

It appears that everyone is expected to do more with less today and in the future the same may be said for swine nutritional research.

Ingredient Matrices

Ingredient matrices used to formulate and design swine feeding programs are dependent upon obtaining analyses that reflect the nutrient values of the chosen ingredients. There may be some economically substantial differences between matrices used in the same regions. One only needs to view some of the more available feedstuffs tables to reach the previously stated conclusion. The variations observed for normal corn and soybean meal are only the beginning. Those variations probably are of minimal concern when they are the only sources of amino acids and energy in swine diets.

The uncertainties begin to surface as one of the newer methods of applying amino acids is used. Formulating diets based on methionine and cystine, threonine, and tryptophan to lysine ratios and allowing up to eight pounds of lysine per ton of diet has made the updating and accuracy of ingredient matrices more critical. The economical differences on a large grow/finish unit can be substantial. As economics supports this method of diet formulation, it will force the industry to critically evaluate nutrient values of the major ingredient inputs.

Another challenging area is the influx of new ethanol plants and the use of distillers' dried grains with solubles. The variation between plants, especially at start-up, can be substantial. Since this is a fairly new ingredient for swine, total nutrient values as well as true or apparent ileal amino acid digestibility values are being gathered, but there are not a lot of data per plant. The new ethanol distillers is one of several co-products that may be available for swine feed in the future.

Struggling to attach meaningful nutrient values to alternative ingredients is not new to our industry. The analyses are generally limited which leaves one in a quandary. Do we supply conservative values and reduce the ingredient's potential value or do we supply aggressive nutrient values to lower usage input costs? If we overvalue the nutrient content of an alternative ingredient, we run the risk of reduced performance by the pig. If we undervalue the nutrient content, we may leave money on the table. The conservative method is probably best in the long run, but there's bound to be some ingredient overvaluing occurring

Ingredient matrices need to be updated as new inputs are available. Gathering analyses on major ingredients is an on-going process. It takes time and money. However, if the analyses are outdated or inaccurate, it will cost the swine and feed industry in the long run. Procedures to gather representative samples, analyze the samples, and publish the results as Degussa Corporation does on a limited basis is a tough task, but of significant value to the industry. The problem is the timeliness of the process and getting adequate samples per region.

Feed Additives

Fear of losing or restricting feed additives has existed for a long time in the feed industry. Some speculated that Carbadox would be pulled shortly after it was cleared for use in the early seventies. Feed additives influence the health of swine and therefore the nutritional status of the pig. The challenge today is to keep the additives that are cleared for swine and to add an additive where needed. This translates into proper usage. Use needs to be scientifically based. Many products have been touted, tested, and researched, as replacements; however, few if any have met the challenge of the feed additives' efficacy in swine.

Regulations

Regulatory pressures are certain to escalate on a local, federal and global basis. Future federal and state regulations could make the feed industry an even more regulated business. The feed industry must deal with regulations influencing pig production (environmental and welfare) and with those laws and regulations covering feed production (FDA, AFCO, other state feed laws, and home land security).

On an environmental basis, it may be more critical to more nearly match a feeding program to an animal's nutrient requirements. This will require a very reliable ingredient matrix as previously mentioned. There may be some animal welfare issues that will dictate nutritional profiles with no regard to economics. We may also see the day when minerals such as zinc and copper diet concentrations will be limited.

The feed industry is adaptable to change and regulation if it makes scientific or economic sense. The feed industry has experience adapting to homeland security issues (currently) and FDA regulations such as the restrictions on bovine animal protein products. However, the additional regulations are still a concern and sometimes an economic burden.

Nutritional Concerns as Viewed by a Swine Veterinarian

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Introduction

The science and technology of feeding livestock for optimal growth and productivity has advanced at a staggering rate during the last several years. One simply has to review the evolution of the National Research Council (NRC) Nutrient Requirements of Swine to observe the changes. In the 1988 version of the NRC, nutritional needs were based upon a static set of minimum nutrient levels. The latest version, published in 1998, uses a modeling approach to establish nutrient requirements. This approach considers variables such as sex, genotype, and environmental factors that affect a pig's nutrient needs. Researchers are working to continually improve upon the models used in the 1998 NRC, and seek to further understand and define how environment, genotype, and other factors influence nutritional needs in our modern systems.

The point of this introduction is to highlight the fact that feeding livestock is an evolving and highly technical endeavor that is soundly based in science. In contrast to livestock feeding, livestock medicine is often referred to as "a science **and** an art." While a competent swine veterinarian should approach problems systematically and with scientific data to support her recommendations, there often comes a time in the life of a problem when the veterinarian must be creative and simply try something new.

It is at this juncture of science and art that the veterinarian and nutritional consultant advising a pork production system may not understand the other's perspective. However, we ultimately do share a common goal: to keep the producer producing wholesome food profitably. The purpose of this paper is to help bridge this gap in perspectives by illustrating a few examples where the paths of the nutrition consultant and the herd veterinarian cross.

Historically, this might have been a presentation about nutritional deficiencies. Our current understanding of swine nutrition, however, has virtually eliminated diseases of deficiency from routine swine practice. Instead, the examples given are illustrations of diseases for which the solution lies in the feed.

Examples

1. Sow Constipation

Clinical observation of constipated sows in gestation stalls and in farrowing crates is not uncommon in our practice. Constipation is not often observed in pen housing, possibly due to increased levels of exercise in penned sows (Hill et. al. 1998). From a veterinarian's perspective, constipation is a problem for both the health and the welfare of the sow. Constipation in the farrowing barn leads to a decrease in lactation feed intake. This may lead to lighter pigs at weaning or increased wean-to-estrus interval.

When a problem with constipation occurs in a sow herd, the easy solution is to add a chemical laxative to the feed. However, observations from practice suggest that desired stool consistency is more difficult to maintain with chemical laxatives, especially with the variable feed intakes associated with lactation. A more predictable response occurs with adding fiber to the diet (Tabeling, et. al. 2003). If fiber is to be added, especially in gestation, rebalancing the ration with an increase in vitamin/mineral premix as well as attention to energy and protein intake is required. Rebalancing the ration and follow-up evaluation of body condition scores is best accomplished by the herd's nutritional consultant.

In addition to predisposing sows to constipation, lack of dietary fiber can have a direct influence on gastric ulcer formation (Straw et. al. 1999). Fiber

in the diet also affects sow welfare by improving sow longevity and reducing stereotypical behavior (Koketsu et. al. 1999). So, it may be less expensive and easier to include a chemical laxative in sow diets, but the additional benefits of added fiber should be considered in the final analysis.

2. Molds and Mycotoxins

In the late summer and early fall of 2004, outbreaks of abortion were observed in three of our clients' herds. One herd was located in north-central Illinois and the other two in northern Indiana. The clinical presentation of these cases was similar in each of the three herds with the first observed clinical sign being 5-15% of gestating sows going "off-feed." Of affected sows, approximately 10% aborted their litters. Rectal temperatures of the affected sows remained normal throughout the course of the outbreak.

One manager reported that the weekend barn crew had observed "large chunks of black, caked feed" when he fed sows on the Saturday prior to the onset of clinical signs. By the time the incident was reported on Monday, wasted feed from the weekend had been discarded.

Of the two other cases, one herd did not have feed available for testing. Tests for molds and mycotoxins from the third herd were negative.

After a 3-5 day clinical course, each case resolved spontaneously. Tests for Porcine Reproductive and Respiratory Syndrome (PRRS) were negative in affected sows in all three herds. Later testing for PRRS and swine influenza virus failed to identify recent infection. In all three cases, mycotoxins were suspected as a likely etiology, but no diagnosis was confirmed.

Veterinarians in our practice recommend routine use of effective mold inhibitors and toxin binders from mid- to late-summer until new corn is available in the fall. Some may argue that this is an unnecessary addition of cost to the rations. From a veterinarian's perspective, however, even one aborted litter is costly and cases like these are not uncommon. We recommend selection of mold inhibitors and toxin binding agents that have low inclusion rates and minimal negative effects on vitamin and mineral availability (Jadamus and Schneider 2002).

3. Enterotoxigenic *E. coli* (ETEC) in Nursery pigs

ETEC continues to be a major cause of morbidity and mortality in nurseries throughout our practice area. While *E. coli* is a normal inhabitant of the swine digestive tract, pathogenic forms are not normally present and can produce devastating disease (Straw et. al. 1999). Several serogroups of ETEC are prevalent in swine populations, causing clinical disease including diarrhea, malabsorption, and acute death (Edema disease).

Our observation in nurseries with clinical ETEC is that signs usually develop after a change in the ration. This typically occurs when pigs that are weaned onto pellets are fed the first ground ration in the nursery. This is not to say that the ration is improperly balanced or is in any way the cause of the problem. However, as an industry, we tend to push production to its maximum, which in the nursery demands frequent diet changes to meet the changing needs of a fast-growing animal. Rapid changes in the diet can disrupt the microflora of the gut in any species. In addition to focusing on the perfect diet for each stage of production, we also need to place a high priority on investigating dietary transitions and the impact that diet changes have on disease.

Veterinarians sometimes recommend zinc oxide at high levels in nursery diets to inhibit colonization of *E. coli*. This recommendation has often been misunderstood by the nutritional consultants working with our clients. The zinc oxide recommendation is not based on the belief that zinc is limiting in the diet. In fact, zinc oxide is poorly absorbed from the pig's digestive tract, and is recommended for its medicinal qualities as a chemical inhibitor of *E. coli* colonization of the intestinal mucosa (Roselli et. al. 2003). When recommending zinc oxide for this purpose, we start with an inclusion rate of 6 pounds per ton of complete feed in the first few ground rations in the nursery.

4. Other Diseases

Several other diseases and problems that we encounter routinely in practice can be addressed, at least in part, by manipulation of the diet. Highly bioavailable forms of zinc such as zinc-methionine

can be used to help prevent Greasy Pig Disease. Cracked hooves and lameness in sows can sometimes be helped by addition of extra biotin to sow rations. Feeding management and feeder type in the farrowing barn can have a great impact on wean-to-estrus interval, due to the effect of feed intake in lactation on body condition in breeding. Inadequate water availability can depress feed intake and milk production in lactating sows.

5. Concern for the Future

In June 2005, the American Academy of Pediatrics, the American Public Health Association, Environmental Defense, Food Animal Concerns Trust, and the Union of Concerned Scientists filed a formal regulatory petition with the Food and Drug Administration (FDA) requesting that the FDA withdraw approvals for routine herdwide use of seven classes of antimicrobials. These seven classes are penicillins, tetracyclines, macrolides, lincosamides, streptogramins, aminoglycosides, and sulfonamides (AVMA 2005). The American Medical Association has formally opposed the use of "non-therapeutic levels" of antimicrobials in livestock feeds since June 2001.

The European Union, Denmark, and other countries have already imposed bans on non-therapeutic use of antimicrobials in livestock feeds. Since their ban, Denmark has observed an increase in antimicrobial use for therapeutic purposes in livestock, an increase in production costs, and an increase in animal sickness and mortality rates (WHO 2002).

The intended purpose of these bans is to reduce the prevalence of antimicrobial-resistant bacteria in human populations. To date, there is no scientific evidence to support this approach to the public health issue of resistant bacterial infections. The political process does not always follow in the footsteps of scientific evidence, however, and American producers may yet find themselves in the same bin as the Danish producers.

This political situation only heightens the need to develop alternatives to feed-grade antibiotic use for growth promotion and disease prevention in our livestock. The industry is looking to nutrition research to provide these alternatives. As feed additive alternatives are developed, the infrastructure to manufacture and distribute these alternatives already exists within the livestock feeding industry.

Conclusions

Some feeds may be better than others at supporting optimal performance, but few (if any) commercially available rations for pigs are unable to support the basic needs of pigs for health and growth. In routine swine practice, nutritional deficiencies are rarely observed, and when they are it is often the result of a feeding or mixing error rather than an inadequate ration.

Many disease processes in pigs are impacted (either positively or negatively) by ingredients in the feed or by feeding management. Nutritional consultants and veterinarians cannot work independently of one another and expect to meet the needs of pork producers, because our disciplines are intertwined. Therefore, it is important that we realize that each consultant approaches a pork production system from a different perspective based on his or her education and experience.

Much of the art associated with feeding pigs has been replaced by science in integrated pork production systems. The art of practicing veterinary medicine, however, still has a significant influence on a veterinarian's recommendations to a pork producer. We share a common goal. As we work with pork producers toward optimal productivity, food safety, and pig welfare, we should not allow different perspectives to become competing opinions.

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Update on Current Issues of Importance for Feed Manufacturers

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Introduction

It is generally recognized that modern feed and food production is a complex global system and what we eat and how it is produced are increasingly scrutinized. This not only includes scrutiny by animal producers and feeders but the final consumers of meat, poultry, and eggs at the restaurant, grocery and other retail outlets. We are constantly reminded of the importance of feed and food safety as we read headlines in trade association updates and other mass media publications. It is of paramount importance that feed manufacturers heighten their concerns expressed and develop quality and other related programs to meet the increasing regulatory oversight requirements. This paper should provide some insight on two activities that dramatically affect national and international food and feed safety.

Strengthen Safeguards Against Bovine Spongiform Encephalopathy (BSE)

Since November 1986 (when BSE was first identified as a separate disease entity), over 178,000 head of cattle have been diagnosed with BSE in Great Britain. The epidemic peaked in January 1993 at approximately 1,000 new cases reported per week. Agricultural officials in Great Britain have taken a series of actions to eradicate BSE, including making BSE a notifiable disease, prohibiting the inclusion of mammalian meat-and-bone meal in feed for all food-producing animals, prohibiting the inclusion of animals more than 30 months of age in the animal and human food chains, and destroying all animals showing signs of BSE and other animals at high risk of developing the disease.

BSE is classified as a transmissible spongiform encephalopathy (**TSE**). The agent responsible for BSE and other TSE's is smaller than the smallest known virus and has not been completely characterized. There are three main theories on the nature of the agent: (1) the agent is a virus with unusual characteristics, (2) the agent is a prion-an exclusively host-coded protein that is modified to a partially protease-resistant form after infection, and (3) the agent is a virino-a small, noncoding regulatory nucleic acid coated with a host-derived protective protein. The BSE agent is extremely resistant to heat and to normal sterilization processes. It also does not evoke any detectable immune response or inflammatory reaction in host animals.

In cattle naturally infected with BSE, the BSE agent has been found only in brain tissue, in the spinal cord, and in the retina. The distal ileum, bone marrow, dorsal root ganglion, and trigeminal ganglion from experimentally infected cattle were also found to be infective. To date, there has been no evidence of infection detected in milk or muscle tissue.

Until December 2003, BSE had not been diagnosed in the United States, and USDA has worked proactively to keep it that way. The dairy cow that first triggered FDA and USDA's emergency action plan was found to be imported from Canada after the feed ban was established. A second confirmed positive cow was found during routine surveillance of high risk animals. The animal was selected for testing because, as a non-ambulatory animal, it was considered to be at higher risk for BSE. In late June 2005, USDA confirmed, by DNA testing, that they had identified the source herd of the animal found to be positive for BSE. Based on information released by USDA, the cow was born and raised in a herd in Texas and was approximately 12 years old. They cow did not enter the food chain; however, it was sent to a pet food plant in Texas and was selected for sampling on arrival.

Safeguards

In cooperation with USDA's Food Safety and Inspection Service (**FSIS**), APHIS has taken stringent measures in prevention, education, surveillance, and

response. To prevent the establishment and amplification of BSE in the country, since 1989 APHIS has prohibited the importation of live ruminants from countries where BSE is known to exist in native cattle. Other products derived from ruminants, such as fetal bovine serum, bone meal, meat-and-bone meal, blood meal, offal, fats, and glands, are also prohibited from entry, except under special conditions or under USDA permit for scientific or research purposes.

On December 12, 1997, APHIS extended these restrictions to include all of the countries in Europe due to concerns about widespread risk factors and inadequate surveillance for BSE.

As of December 7, 2000, USDA prohibited all imports of rendered animal protein products, regardless of species, from Europe. This decision followed the recent determination by the European Union that feed of nonruminant origin was potentially cross-contaminated with the BSE agent. The restriction applies to products originating, rendered, processed or otherwise associated with European products. USDA has taken emergency action to prevent potentially cross-contaminated products from entering the United States. The same type of rendered product from ruminant origin has been prohibited from BSE-infected countries since 1989.

APHIS leads an ongoing, comprehensive, interagency surveillance program for BSE in the United States. BSE is a reportable disease by accredited veterinarians. APHIS veterinary pathologists and field investigators have received training, including training from their British counterparts in diagnosing BSE.

The surveillance samples include field cases of cattle exhibiting signs of neurological disease, cattle condemned at slaughter for neurologic reasons, rabies-negative cattle submitted to public health laboratories, neurologic cases submitted to veterinary diagnostic laboratories and teaching hospitals, and sampling of cattle that are nonambulatory (downer cattle/fallen stock).

FDA's Enforcement Plan of the Ruminant-to-ruminant feed ban

As an additional preventative measure, the Food and Drug Administration's (FDA) promulgated regulation (effective August 4, 1997) prohibiting the use of most mammalian protein in the manufacture of animal feeds given to ruminants. In addition, the final regulation also requires process and control systems to ensure that ruminant feed does not contain the prohibited mammalian tissues.

FDA's enforcement plan for the ruminant feed regulation includes education, as well as inspections, with FDA taking compliance actions for intentional or repeated non-compliance. The state regulatory agencies conduct over 70% of the inspections for FDA. To date, states have conducted 25,000 inspections of renderers, feed mills, ruminant feeders, protein blenders, pet food manufacturers, pet food salvagers, animal feed distributors and transporters to determine compliance with the BSE/Ruminant Feed regulations.

The FDA has also drafted an emergency response plan to be used in the event that BSE was identified in United States. This plan was put into action on December 23, 2003 when a single dairy cow was identified in Washington state as being positive for BSE.

On July 9, 2004, Human Health and Services Secretary Tommy G. Thompson and Agriculture Secretary Ann M. Veneman announced actions being taken to further strengthen existing safeguards that protect consumers against the agent that causes bovine spongiform encephalopathy (BSE, also known as "mad cow disease"). To allow interested parties and stakeholders the opportunity to comment on the additional regulatory and policy measures under consideration, USDA's APHIS and FSIS, along with the FDA, developed an advance notice of proposed rulemaking (ANPRM) that includes several additional actions the federal government is considering regarding BSE. The advance notice of proposed rulemaking will allow the public the opportunity to provide their input.

The ANPRM also requested comment on the following measures related to animal feed, which is regulated by FDA:

1. removing specified risk materials (SRM's) from all animal feed, including pet food, to control the risks of cross contamination throughout feed manufacture and distribution and on the farm due to misfeeding;

2. requiring dedicated equipment or facilities for handling and storing feed and ingredients during manufacturing and transportation, to prevent cross contamination; and

3. prohibiting the use of all mammalian and poultry protein in ruminant feed, to prevent cross contamination; and prohibiting materials from non-

ambulatory disabled cattle and dead stock from use in all animal feed.

FDA has reached a preliminary conclusion that it should propose to remove SRM's from all animal feed and is currently working on a proposal to accomplish this goal.

Actions in Response to BSE

FDA's enforcement plan for the ruminant feed regulation includes education, as well as inspections, with FDA and state agencies taking compliance actions for intentional or repeated non-compliance. State regulatory agencies conduct over 70% of the inspections for FDA under contract, letter of memorandums, or other informal agreements. Over 36 states contracts annually with FDA to conduct a specific number of BSE and Good Manufacturing Practice inspections of feed manufacturers, ingredients suppliers, and allied industries across the country. The most recent inspection data for all firms inspected can be found on FDA's database website (http://www.accessdata3.fda.gov/BSEInspect/).

The compliance rate of all firms with the BSE regulation is extremely high and the number of firms in compliance has increased over the last six to seven years. I strongly believe that the early educational and inspectional efforts of regulatory agencies play a large role in the high compliance rate with the regulation. The number of firms handling prohibited proteins has significantly decreased along with the number of products containing prohibited proteins during the past five years. At least in the Commonwealth of Kentucky, the majority of the Kentucky firms handling and processing prohibited proteins are the renderers, single-specie feed processing facilities or pet food manufacturers.

Animal Feed Safety System Update

Feed safety in the U.S. has been a topic of conversation among industry trade groups and regulatory agencies. The common theme during these conversations is the need for oversight programs to ensure the production of safe feed. The Association of American Feed Control Officials (**AAFCO**) and the Food and Drug Administration are exploring the development of model feed safety programs. AAFCO has published their Best Management Practices Document which is a voluntary program developed with cooperation with their industry advisors. In 2003, FDA announced plans to develop an Animal Feed Safety System (**AFSS**) that would cover a wide spectrum of feed production including ingredient suppliers, non-medicated feed manufacturers and the transportation sector.

The proposed AFSS is still in the developmental stages and is being revised by the committee established by the FDA to explore the options. During the most recent public meeting, held a public meeting on April 5-6, in Omaha, NE, the Center for Veterinary Medicine (**CVM**) discussed the committee developments to date. The focus of CVM's second public meeting was to discuss the proposed draft framework that described the features FDA believes should make up the feed safety system.

The draft framework, which covers all aspects of feed production, was published in February 2005 and it identified four components that will make up the feed safety system. The components explained below outline the purpose, goal and identified gaps within each component.

• ***Component 1: Ingredients and the Approval Process.*** The purpose of this component of the feed safety system requires that all ingredients used in feed are safe. This component also describes the mechanisms FDA and CVM use to make sure all ingredients and additives used in feed are safe for the uses intended. The principal mechanism is the Federal Food, Drug, and Cosmetic Act. However, FDA has also relied on the Association of American Feed Control Officials to define ingredients. The gap identified under AFSS for this component is that a non-Federal organization is used to list ingredients and provide information. The framework document identifies the use of an FDA Compliance Policy Guide to correct the gap.

• ***Component 2: Limits for Animal Feed Contaminants.*** The purpose of this component is to identify the hazards that feed might contain and set limits to those hazards. In addition, this component calls for developing test methods to find the hazards. One gap the AFSS team identified is the lack of a ranking process that would allow FDA to determine which hazards require limits and analytical methods. CVM is developing a risk assessment method, which was also discussed at the meeting.

• ***Component 3: Process Control for the Production of Safe Feed.*** This component deals with proper manufacture, packaging, storage, and distribution of

feed ingredients and mixed feed to keep hazards out. Current FDA regulations cover medicated feed manufacturers. However, under AFSS, FDA is exploring a broader regulatory approach to cover production, packaging, storage, distribution, and use of feed ingredients and non-medicated feeds.

• ***Component 4: Regulatory Oversight.*** This part of the feed safety system calls for regulators to apply a risk-based system so that FDA can use resources for the greatest benefit in terms of keeping feed safe. One gap that the framework identified is that some segments of the feed industry, including transporters and on-farm mixers, are outside the normal regulatory scope of FDA and the States.

Additional information and updates on the progress of the proposed Animal Feed Safety System can be found on FDA's website located at http://www.fda.gov/cvm/ The public is welcome to make comments on the proposed framework document. Written comments should be sent to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. Submit electronic comments to http://frwebgate.access.gpo.gov/cgi-bin/leaving.cgi?from=leavingFR.html&log=linklog&to=http://www.fda.gov/dockets/ecomments.

Conclusion

The importance of feed safety for interstate commerce and international trade is increasing in importance for not only feed manufacturers, feed distributors and regulatory agencies. Although the feed industry is being proactive, consumers of meat, milk, and eggs are in the forefront and active in food safety related issues. It is the author's belief that if feed manufacturers want to remain competitive in the feed trade it will be of paramount importance for them to adapt to these changes and develop quality and feed safety programs that address the heightened concerns. The preferred approach to the development and implementation is subject to one's perspective; however, from my perspective these programs are best accomplished through early educational and inspectional efforts by regulatory agencies along with assistance and input from all interested stakeholders.

Amino Acid Nutrition of Swine: Fifteen Vexatious Questions

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Summary

Fifteen provocative questions concerning amino acid nutrition are posed in this review. The last three questions refer to important new information that has direct application to amino acid nutrition of swine. Some form of the ideal protein concept is being used for feed formulation in swine throughout the world. New information is available on ratios for maintenance *per se* and protein accretion *per se* and this allows more accurate estimates of ideal amino acid ratios for grow-finish pigs at each stage of growth. Both threonine and sulfur amino acids have lower ideal ratios than previous estimates would indicate. For gestation, new research suggests that the requirement for lysine is over twice as great (g/d) during d 70 to 114 as for d 0 to 70 of gestation. Also, the ideal ratio of dietary threonine:lysine is projected to decrease considerably between d 0 to 70 and d 70 to 114 of gestation. For lactation, the threonine:lysine ratio is vastly different for high feed intake - low weight loss sows vs. low feed intake – high weight loss sows. Lactating sows losing 33 to 45 kg during a 21-d lactation have an estimated dietary threonine:lysine ideal ratio of 69%, but sows losing no weight during the same period (i.e., no milk amino acids coming from tissue mobilization) require a threonine:lysine ideal ratio of only 59%.

Introduction

This (unconventional) paper will focus on 15 questions that have intrigued me over the course of my career. Short answers (or suggestions) together with some key references will be given to most of the questions, but newer information on ideal amino acid (AA) ratios for grow-finish, gestating and lactating swine will be discussed in more detail. The short answers to some of the questions will emphasize only a few key references, many of which are my own. More detail on the body of work that has been done can be found in the papers cited.

Fifteen Questions

Q1: Why Is Protein Accretion Increased When Methionine Is Added to a Protein-Free Diet?

Numerous reports have indicated that methionine (Met) addition to a protein-free diet reduces body weight loss and improves protein accretion in rats, chicks, pigs and dogs. Webel and Baker (1999) showed that the Met response was due to Met furnishing cysteine. Thus, the first-limiting AA for endogenous protein synthesis must be cysteine.

Q2:Why Is Excess Dietary Cysteine So Much More Noxious than an Equivalent Excess of Cystine or N-acetylcysteine?

Although L-cysteine, L-cystine, and N-acetyl-L-cysteine are equally efficacious on a molar basis for protein accretion and growth (Baker, 2006), they are far different when fed at pharmacologic dose levels, i.e., levels providing cysteine at 5 times (or greater) the requirement (Harper et al., 1970; Silk et al., 1974; Baker and Czarneck-Maulden, 1987; Dilger and Baker, 2005; unpublished data). Addition of 3 or 4% L-cysteine to a typical corn-soybean meal diet for chicks or rats causes heavy mortality within 5 d

of assay initiation. Similar levels of L-cystine or N-acetyl-L-cysteine (or Met, for that matter) result in no mortality after 10 d of feeding. Cysteine is absorbed from the gut faster than cystine (Silk et al., 1974), and it has potent reducing-agent activity as well as mineral-chelation activity (Baker and Czarnecki-Maulden, 1987). It can also bind plasma proteins (Baker, 2006). N-acetylcysteine is less toxic than cysteine, perhaps because the deacetylation process occurs more slowly. This is fortunate in that N-acetylcysteine is being used increasingly in the clinical setting (Kelly, 1998). It, along with cysteine itself, is also available over-the-counter in both health-food stores and pharmacies. It shouldn't be!

Q3:Why Is Cyst(e)ine the Least Digestible Amino Acid in Protein Sources Fed to Swine?

Virtually all protein sources used in swine feeds have undergone some form of heat treatment, i.e., drying, heat processing, pelleting. This causes a significant portion of protein-bound cysteine to be oxidized to cystine, and protein-bound cystine is less digestible than protein-bound cysteine (Miller et al., 2001). Thus, dietary cyst(e)ine (i.e., cysteine + cystine) has a low digestibility (Parsons et al., 1992).

Q4: Why Are Growth Responses to Methionine Often Nonlinear?

Many studies employing graded dosing of Met have involved Met-deficient basal diets that either have a marginal excess or a marginal deficiency of bioavailable cyst(e)ine. In the former case, Met additions to the diet increase protein accretion (at some point(s) on the response curve) due to (a) provision of Met and (b) Met allowing a portion of the dietary cyst(e)ine to be used for protein synthesis. In the latter case, Met is furnishing both Met and cysteine, and when Met furnishes cysteine it is only 81% efficient on a wt:wt basis (Dudley-Cash and Baker, 2004; Baker, 2006).

Q5: What Is the Relevance of S-Methylmethionine In Nonruminant Nutrition?

Feedstuffs used for swine contain significant quantities of S-methylmethionine (SMM); soybean meal (SBM), for example, contains 0.17% SMM (Augspurger et al., 2005). Because SMM is an analog of S-adenosylmethionine, it is capable of replacing this compound in biological methylation reactions, e.g., choline synthesis from phosphatidylethanalamine, creatine synthesis from quanidinoacetate. Biosynthesis of Met from homocysteine, however, will use SMM only under conditions of dietary choline (or betaine) deficiency (Augspurger et al., 2005). Thus, SMM can spare the dietary choline requirement, but not the dietary Met requirement (i.e., unless choline/betaine is deficient in the diet).

Q6: What Are the Priorities of Use When an Amino Acid With Multiple Functions Is Deficient?

Clearly, AA are used to synthesize a variety of different body proteins (e.g., soft-tissue, acute-phase, keratoid, hormonal, and specialized proteins such as metallothionein). Also, some AA have precursor roles. Robbins et al. (1977) showed that histidine's priority was for protein synthesis rather than carnosine synthesis, and Chung et al. (1990) demonstrated that protein synthesis is prioritized over glutathione synthesis when cysteine is deficient in the diet. Concerning the synthesis priority of one type of protein over another, little is known about this intriguing question.

Q7:Why Is It When a Diet Is Equally Deficient in an Amino Acid and Two Different B-Vitamins That Growth Will Respond Markedly to Dietary Addition of Any One of the Three Deficient Nutrients?

In underdeveloped countries, poor nutrition is characterized by multiple nutrient deficiencies. We developed a soy-protein isolate basal diet that could be made markedly deficient in several essential nutrients, e.g., Met, choline, riboflavin, vitamin B-6 and Zn (Baker et al., 1999). Surprisingly, when diets were made approximately equally limiting in any pairs or trios of these nutrients, marked growth responses were found to occur from any one of the deficient nutrients. Thus, the order of limiting AA concept in which responses will not occur to a 2nd or 3rd limiting AA unless the 1st (or 1st and 2nd) limiting AA is supplemented does not apply when multiple deficiencies of AA, vitamins, and trace minerals exist in a diet (Baker et al., 1999). Logical explanations for this phenomenon are not obvious.

Q8: Why Does a Deficiency of One Amino Acid Produce Different Results Than an Equal Deficiency of Another (Different) Amino Acid?

All single AA deficiencies also involve a profile of excess AA over and above the single deficiency – and each single deficiency results in a unique and different profile of excess AA. The excess AA can have very different effects on voluntary food intake, depending on which specific AA is deficient. Using a crystalline AA diet, Sugahara et al. (1969) evaluated single deficiencies (60% of required level) and compared them to a deficiency of all essential AA (i.e., all at 60% of required level). Single deficiencies of phenylalanine + tyrosine, tryptophan (Trp) or isoleucine resulted in poorer growth (due to lower food intake) than that which occurred from a deficiency of all AA together. The excess AA over and above each deficiency, although having variable effects on voluntary food intake, did not have negative effects on food efficiency, i.e., relative to the deficiency of all AA. Why certain excess AA profiles cause food intake reduction while other profiles do not remains a mystery.

Q9: To What Extent Does Gut Synthesis of Essential Amino Acids Contribute to the Amino Acid Requirements of Pigs

Torrallardona et al. (2003) used ^{15}N and ^{14}C labeling experiments to evaluate gut AA biosynthesis and subsequent ileal absorption in 20 kg pigs. Amino acid absorption of microbial origin was estimated at 1.1 g/d for lysine (Lys), 2.0 g/d for leucine, 1.8 g/d for valine, and 0.8 g/d for isoleucine. These quantities are not insignificant. In fact, they exceed the estimated maintenance dietary needs for these AA. Thus, the true maintenance requirements for AA must be the sum of true ileal digestible (TID) dietary needs plus the amount provided by gut microbial synthesis. For a 20 kg pig, this would make the total TID Lys maintenance requirement 1.4 g/d rather than the TID dietary Lys maintenance requirement of 304 mg/d (Heger et al., 2002).

Q10: Why Does a Large Excess of Dietary Lysine Elicit a Growth Response in Niacin-Deficient Animals?

Niacin activity comes not only from ingested niacin (or niacinamide) but also from ingested Trp. About 95% of the Trp flux during turnover goes to CO_2 (via α-ketoadipic acid), 3% to serotonin, and a 2% to nucleotides of nicotinate. α-ketoadipic acid is also an intermediate in Lys catabolism to CO_2. Thus, Augspurger and Baker (2003) showed that addition of 1 to 1.5% excess Lys to a niacin-deficient diet elicits a growth response in chicks. The same Lys additions to a niacin-adequate diet causes substantial growth depressions. At the key branchpoint of Trp catabolism to either niacin nucleotides or CO_2 (i.e., at 2-amino-3-carboxymuconic acid semialdehyde) α-ketoadipate is projected to accumulate due to Lys catabolism. We suggest that this forces more of the 2-amino-3-carboxymuconic acid semialdehyde flux in the direction of niacin nucleotide synthesis, with less being directed to CO_2 via α-ketoadipic acid.

Q11: Why Are 10 to 50% of Absorbed Amino Acids Wasted (Catabolized) When Fed to Growing Animals Well Below Required Levels for Maximal Protein Accretion?

Numerous studies have now verified what might be referred to as the inefficiencies of AA use for protein accretion (Chung and Baker, 1992; Batterham, 1994; Adeola, 1995; Heger et al., 2002; 2003; Baker, 2004). Thus, at well below required levels, AA recovered in whole-body protein represent only 50 to 90% of the AA fed (i.e., absorbed, since the AA fed are crystalline AA or derived from highly digestible casein). The AA that stands out as being the most inefficiently utilized is Trp. Over 50% of absorbed Trp cannot be recovered in whole-body protein. Work in this area also suggests that the efficiencies of utilization for each essential AA are constant at all levels of intake between maintenance and about 90% of the requirement for maximal protein accretion.

Q12: Can Maternal Diet Affect Sex Ratio of Offspring?

Rosenfeld et al. (2003) and Rosenfeld and Roberts (2004) fed (*ad libitum*) female mice a diet either high in saturated fat (lard) or a diet low in saturated fat but high in carbohydrates from 4 to 45 wk of age. A total of 1,048 offspring were born from 108 pregnancies. Sex ratio of offspring was close to 1:1 for dams bred at 10 wk of age, regardless of maternal diet. However, sex ratio of offspring for dams bred at 20, 28, or 40 wk of age was 0.67:0.33 (male: female) for dams fed the high-fat diet. Conversely, in mature dams fed the low fat-high carbohydrate diet,

the sex ratio of offspring was skewed toward females (0.39:0.61 male:female). Explanations for these fascinating observations have been proposed but not empirically tested. Krüger et al. (2005), however, in their 30-yr evaluation of sex ratio in springbok (an African antelope), suggest that sex ratio determination is most likely to occur at or near the time of embryo implantation. One wonders whether sex-ratio skewing due to diet could occur in mature sows, and if so, how long the feeding period would need to be to effect the change.

Q13: Is There New and Accurate Information on Amino Acid Requirements and Ideal Ratios for Swine Maintenance? For Protein Accretion?

Heger et al. (2002; 2003) in the Czech Republic has completed an extensive series of experiments in which graded levels of each essential AA were fed to 44-kg pigs. Amino acid-fortified casein diets were used to assure near complete absorption of AA. Nitrogen retention was regressed on AA intake to establish linear regression equations for each AA. This allowed calculation of both maintenance requirements (zero N retention) and rates of AA uptake into whole-body protein (i.e., mg AA per g protein). From these studies, Heger et al. (2002; 2003) were able to calculate ideal AA ratios for maintenance *per se* as well as for protein accretion *per se*. These together with the NRC (1998) estimated ratios are shown in Table 1. Accretion ratios for threonine (Thr), SAA and Trp estimated by the Czech workers are lower than those estimated by NRC (1998), whereas maintenance ratios for Thr and SAA are lower and Trp higher for the Heger et al. (2002; 2003) estimates than for the NRC (1998) estimates. Also, the isoleucine maintenance ratio of Heger and coworkers is much lower than the maintenance isoleucine ratio estimated by NRC (1998). Because empirical evidence is now available on isoleucine requirements of pigs, I have suggested isoleucine ratios for both protein accretion and maintenance that differ from both the NRC (1998) and Heger et al. (2002; 2003) estimates.

To use the data in Table 1 to estimate overall ratios (i.e., protein accretion plus maintenance), it is helpful to have an estimate of the maintenance contribution to the overall requirement, and what is therefore important is the relative difference between accretion and maintenance ratios. For most AA, the differences are not great, but for Thr, SAA, and Trp, maintenance ratios are over twice as high as accretion ratios. Using NRC (1998) requirement estimates (g/d for high-lean pigs) for the AA shown in Table 1 together with the maintenance requirement estimates of Heger et al. (2002; 2003), except for isoleucine, one can estimate the maintenance contributions to the total requirement for each AA. These values are shown in Figure 1, and for simplicity, they are shown as straight-line functions of body weight. Also, Thr, SAA, and Trp are considered together and are distinguished from all the other AA, which are also considered together. It should be noted that the principal problem in these calculations is the accuracy of the NRC (1998) requirement estimates for AA other than Lys. In particular, NRC (1998) Thr requirements may be overestimated and Trp requirements underestimated for older pigs if the NRC (1998) maintenance ratio for Thr is too high and that for Trp too low, as suggested by the Czech results. Nonetheless, the maintenance contribution to the total requirement for Thr, SAA, and Trp is predicted to increase from averages of about 6% to 14% for pigs in the 10 to 20 kg and 80 to 120 kg weight ranges, respectively. For all other AA, including Lys, the maintenance contribution is lower, increasing from an average of about 3% (10 to 20 kg) to an average of about 6% (80 to 120 kg).

Table 2 shows overall predicted ratios at four different body weight ranges, using the information provided in Table 1 and Figure 1. The ratios for most AA are similar to those predicted earlier (Baker, 1997), but those for SAA and Thr are lower at all weight ranges, and the ratios for phenylalanine + tyrosine and leucine are higher at all weight ranges. The overall ratios for Thr and SAA in Table 2 are also somewhat higher than those calculated by Heger et al. (2003) for pigs weighing 20, 50, and 100 kg. The empirical evidence (Chung and Baker, 1992) provided to support the ratios proposed originally (Baker, 1997) validated that the proposed ratios (in all cases equal to or lower than the Fuller et al. (1989) ratios) may not represent minimums. It appears based on the new information from Heger et al. (2002; 2003) as well as recent requirement information that previous ideal ratios for Thr and SAA were too high, particularly for younger pigs. It is my view that the ideal AA ratios shown in Table 2 are more accurate than those predicted previously by Fuller et al. (1989), Baker (1997), or NRC (1998).

Q14: Why Do Ideal Amino Acid Ratios and the Order of Limiting Amino Acids Change Between Early and Late Gestation, and Between Low Feed Intake – High Weight Loss Sows and High Feed Intake – Low Weight Loss Sows?

Gestation. For gestation, AA are needed for (a) sow maintenance, (b) growth of fetal tissue, (c) growth of the mammary gland, and (d) growth of all other maternal tissues. By serial killing methodology, Kim et al. (2005) have estimated that the TID Lys requirement for these functions between d 0 and 70 is markedly lower (6.8 g/d) than for d 70 to 114 of gestation (15.3 g/d) in first-parity sows. If this calculation is correct, why do we feed only one diet that provides 10 g/d of TID Lys throughout the entire gestation period? If 2.0 kg/d is fed throughout gestation, a corn-SBM diet with only 9.8% CP would meet the Lys need for d 0 to 70 of gestation, whereas a corn-SBM diet with 16.6% CP would be needed to meet the Lys requirement for late gestation, i.e., d 70 to d 114. This type of gestation feeding regimen has never been tested, although, qualitatively, Pettigrew and Yang (1997) suggested previously that AA requirements in late gestation should exceed those in early gestation. Their suggested magnitude of difference between early and late gestation, however, was not as great as that suggested by Kim et al. (2005).

Do ideal AA ratios differ between earlier (i.e., d 0 to 70) and later (i.e., d 70 to 114) gestation? Kim et al. (2005) suggest that they do, and the most important change suggested occurs with Thr. Although Kim et al. (2005) likely overestimated the Thr:Lys ratios (they used NRC estimates for Thr maintenance requirements, which are too high), they nonetheless predicted that the ideal Thr:Lys ratio would decrease as gestation progresses.

The Lys requirements suggested by Kim et al. (2005) for early and late gestation do not account for animal-to-animal variability. Nonetheless, the 2.25-fold difference between estimated Lys requirements during d 0 to 70 and d 70 to 114 should be tested empirically, particularly since current gestational feeding practices often lead to low feed intake and high body weight loss of sows during lactation (Mahan and Mangan, 1975; Kim et al., 2005; Soltwedel, 2005). To account for variability from sow to sow, my suggestion would be to test the efficacy of two dietary gestational feeding regimens: (a) 13.3% CP corn SBM diet (0.54% TID Lys) at 2 kg/d, providing 11.1 g/d TID Lys throughout gestation, and (b) 11% CP diet (0.40% TID Lys) at 2 kg/d providing 8 g/d TID Lys from d 0 to d 70 of gestation; and 17% CP diet (0.80% TID Lys) at 2 kg/d, providing 16 g/d TID Lys from d 70 to d 114 of gestation. Regimen (a) results in overfeeding Lys during d 0 to 70, and underfeeding Lys during d 70 to 114. Both regimens, however, provide the same quantity of Lys during the 114-d gestational period, but Regimen (b) is designed to provide Lys more in line with the actual needs of the sow and her developing fetuses as pregnancy progresses. Would feeding protein and Lys in this manner to adequately meet both maternal and fetal needs result in higher feed intake and lower weight loss during the subsequent lactation?

Lactation. Kim et al. (2001) suggested that ideal AA ratios for lactating sows change as a function of sow body weight (and body protein) loss. The most important change occurs in the Thr:Lys ratio. Relative to the Thr need for milk production and mammary gland growth, Thr provided by maternal protein depletion is deficient. Thus, in low feed intake – high weight loss sows, Thr becomes a potentially key limiting AA. In fact, Kim et al. (2005) estimated that the ideal Thr:Lys dietary ratio may need to be as high as 75% for sows losing 75 to 80 kg during a 21-d lactation. This amount of weight loss would be consistent with a 50% contribution of maternal tissue protein catabolism to milk AA output. With minimal 21-d weight loss (0 to 8 kg), the ideal Thr:Lys dietary ratio was predicted to fall to only 60%.

With extreme (75 to 80 kg) weight loss, Thr may become the 1st-limiting AA in a typical corn-SBM lactation diet (0.90% Lys), but in all other weight loss scenarios, Lys is predicted to be 1st limiting (Kim et al., 2005). It is also possible that valine (instead of Thr) may become 2nd limiting after Lys when lactation weight loss is minimal.

Soltwedel (2005) produced the equivalent of a 21-d lactation weight loss of about 25 kg by feeding a low protein diet (10% CP, 0.55% TID Lys). A 17.2% CP corn-SBM diet (0.90% Lys) was diluted (with starch) to achieve the 10% CP diet, and this therefore maintained the same AA pattern that was present in the higher protein diet. Using plasma urea nitrogen as a criterion of AA deficiency, Soltwedel (2005) found that his sows, who likely were relying on tissue protein catabolism for about 30% of the AA output in milk, would respond to Lys (1st limiting) and then Thr (2nd limiting). Valine addition to the Lys and Thr fortified diet, however, did not elicit a response. The important message from these recent studies is that

ideal dietary AA ratios for lactation can change considerably depending on the extent of protein depletion in the sow.

Q15: Is the Protein Quality of Sow's Milk Ideal for Maximal Growth of the Nursing Pigs?

Mavromichalis et al. (2001) observed that the digestibility of some AA in sow's milk, particularly Thr and cyst(e)ine were considerably less than 100%. Also, only 42% of the TID SAA were present as cyst(e)ine, an indication that nursing pigs would have to use some of the ingested Met to furnish cyst(e)ine. True ileal digestible arginine in sow's milk was only 65% of TID Lys, and Wu and Knabe (1994) found even lower concentrations of arginine (relative to Lys) in sow's milk than the values reported by Mavromichalis et al. (2001).

Kim et al. (2004) speculated that because sow's milk is low in arginine, and also because arginine biosynthesis in young pigs is inefficient (Wu, 1997), baby pigs on an artificial milk formula (simulating sow's milk) might respond to arginine supplementation. They reported a 28% growth response from d 7 to d 21 of life from adding 0.20% arginine to the formula; the growth response from 0.40% arginine addition was 65%. Plasma urea nitrogen decreased linearly, and plasma insulin and growth hormone increased linearly in the piglets as a function of arginine supplementation. The implications of this work are profound in that the results suggest sow's milk is markedly deficient in arginine for optimal growth of the nursing piglets. The formula used by Kim et al. (2004), however, contained a level of arginine similar to that present in sow's milk as reported by Wu and Knabe (1994), but this level of arginine was only about 50% as high as that reported by Mavromichalis et al. (2001) as well as by several other investigators (e.g., King et al., 1993).

One could also question whether L-cysteine or N-acetyl-L-cysteine supplementation might elicit a response, although the (usable) SAA:Lys ratio (TID basis) in sow's milk (54.5%) would suggest otherwise (cf. Table 2). However, if transsulfuration efficiency is not fully developed in young piglets, the low TID cyst(e)ine level in sow's milk may suggest that not only arginine, but possibly cyst(e)ine as well, may be important in providing optimal AA nutrition for neonatal piglets.

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Table 1. Ideal ratios for protein accretion and maintenance in grow-finish pigs[a]

	Protein Accretion		Maintenance	
Amino Acid	NRC (1998)	Heger et al. (2002; 2003)	NRC (1998)	Heger et al. (2002; 2003)[b]
Lysine	100	100	100	100
Threonine	60	58	151	126
SAA	55	46	123	118
Tryptophan	18	15	26	41
Valine	68	69	67	59
Isoleucine	54	56 (60)[c]	75	46 (57)[c]
Leucine	102	105	70	85
Histidine	32	33	32	36
Phe + Tyr	93	113	121	110

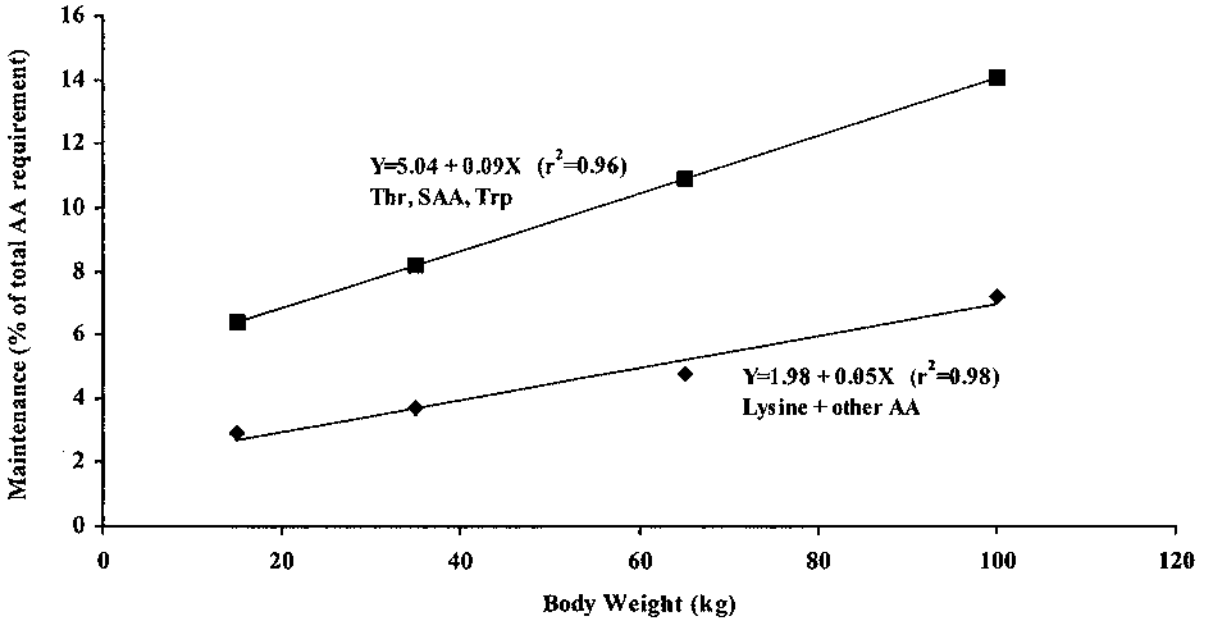
[a]True ileal digestibility basis; % of Lys
[b]Heger et al. (2002) determined the Lys maintenance requirement to be 39 mg/d/kg$^{3/4}$ for pigs weighing 44 kg.
[c]Baker (2005) value

Table 2. Ideal ratios for grow-finish pigs at different periods of growth[a]

	Body Weight (kg)			
Amino Acid	10-20	20-50	50-80	80-120
Lysine	100	100	100	100
Threonine	62	63	65	68
SAA	50	52	54	56
Tryptophan	16.5	17	18	19
Valine	69	69	68	68
Isoleucine	60	60	60	60
Leucine	104	104	103	103
Histidine	33	33	33	34
Phe + Tyr	113	113	113	113

[a]True ileal digestibility basis and based on ratio values in Table 1 (using Heger et al., 2002; 2003), except for isoleucine where the Baker (2005) values were used. The graph in Fig. 1 was used to calculate maintenance and protein accretion contributions to the overall ratios.

Figure. 1. Maintenance contribution to TID requirement for amino acids



Modeling Calcium and Phosphorus Requirements for Growing and Finishing Pigs

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Summary

Phosphorus (P) is widely recognized as a potential environmental pollutant associated with confined swine operations. One way to reduce the total P released into the environment is to feed dietary levels that more closely match the pig's actual requirement. The mathematical modeling, or prediction, of requirements for nutrients has become a technique widely used to establish requirements that can be adaptable to pigs at various stages of growth and with varying genetic backgrounds. Two primary factors influence the total P requirement of a growing pig: P needed for maintenance and P deposited in body tissues. Although calcium (Ca) is not regarded as an environmental pollutant, the same approach can be used to estimate dietary requirements for this nutrient also. This review will discuss studies conducted in pigs to estimate endogenous P losses reflective of a maintenance P requirement, data describing the true digestibility of P and Ca and varying body weights, as well as studies estimating the retention of Ca and P in whole body tissues of growing and finishing pigs

Introduction

In the estimation of nutrient requirements for pigs at various stages of growth and production, two methods can be employed to ascertain the proper feeding level in the diet. The predominant method, as seen in literature reviews of nutrient requirements, has been the empirical method. This methodology allows for the precise collection of response criteria corresponding to the nutrient in question. However, this method is limited in many regards, most notably by the inability to confidently apply the data to pigs of varying genetic backgrounds reared in different environmental conditions. This has led to a more recent insurgence of nutritionists estimating nutrient requirements for pigs using a factorial, or modeling, approach in which an attempt is made to quantify all of the factors that can influence the requirement for a given nutrient. This process was most notably utilized to estimate daily and dietary amino acid requirement levels for swine in the current *Nutrient Requirements of Swine* (NRC, 1998). For growing pigs, factors including lean growth rate, sex, environmental temperature, and crowding conditions are used to estimate dietary amino acid requirements. The flexibility of such a model for nutrient requirement estimation is incomparable, but like most scientific endeavors, is only as strong as the experimental procedures and analysis of data on which it is built.

Phosphorus has quickly become the primary focus of concerns related to the effects of swine production systems on the environment. With the increased concern, attention is focused on nutritional means to reducing P output from the pig. Improving P utilization from the diet by feeding highly digestible P sources and increasing the efficiency of P digestibility with the addition of phytase to swine diets has made large improvements in reducing the excretion of P; however, potentially greater improvements can still be made. Often overlooked in the list of potential solutions to reducing P excretion is to formulate diets that more closely meet the true needs of the pig during each specific stage of production. To assume that the current estimates of dietary P requirements are accurate and unchanging, overlooks the biological aspects of the pig related to P metabolism.

One approach to estimating P requirements for growing pigs is to partition the net requirement into

quantifiable factors, that when summed, estimate the net P requirement for the pig. These factors would include the digestibility of P from various feed sources, P required to replace inevitably lost P from the body, and P retained in whole body tissues as the pig grows. In the current (10th) edition of the National Research Council's *Nutrient Requirements of Swine* (1998), the estimation of P requirements for growing pigs is based on a compilation of empirical studies utilizing growth performance responses to increased levels of P in the diet. The authors of the same publication were able to develop a comprehensive model allowing for the prediction of amino acid requirements based on the factorial approach. Such a system allows for the flexibility of accounting for environmental, genotype, and gender effects on growth and composition of pigs - all influences on the requirements for amino acids. Enough data were not available at the time the NRC publication was written to allow for the same type of approach to estimate P requirements. The information presented here may serve as the initial step in allowing for a factorial model to predict net P requirements in growing and finishing pigs.

Maintenance Phosphorus

The prediction of the net P requirement for growing and finishing pigs begins with the estimation of the daily P required to replace the endogenous loss of P from the body. The term "inevitable" P loss is commonly used to describe endogenous loss; however, this term would, by definition, include the loss of P via feces and urine. It is well understood that P homeostasis in the body is controlled by renal reabsorption when P intake is low, and therefore P excreted via urine when P intake is adequate may not truly reflect an endogenous loss of P that would need to be replaced by the pig, but rather the excretion of excess P beyond the requirement. A pure maintenance requirement for P should be defined as the endogenous P lost during the course of consuming and digesting a given diet that must be replaced to maintain P homeostasis in the body.

In the process of digestion in the gastrointestinal tract of swine, a multitude of enzymes and secretions are utilized for the breakdown of dietary components into forms capable of being absorbed into the body. Because P is a component of all living cells, the passage of these digestive secretions through the gut and out of the body in the feces represents a loss of P that must be replaced. Additionally, secretion of phosphates directly into the large intestine has been observed as a source of P loss from the body (Partridge, 1978). The need for the pig to consume at least this level of P to maintain normal body functions would indicate the existence of a maintenance P requirement.

Some nutritionists would claim that the need for maintenance P is only a theoretical manipulation of the biological processes involved in P balance in the body. To claim that all losses of P would need to be immediately replaced by the animal by increasing intake ignores the role that homeostatic resorption of P from bone plays in maintaining P balance in the body. Additionally, under adequate intake levels of P from the diet, urinary excretion of P is elevated, yet when intake of P is reduced to suboptimal levels, urinary reabsorption of P occurs to prevent too much P from leaving the body (Jongbloed, 1987). Therefore, the conditions requiring any additional input of dietary P to replace inevitable P losses would only occur if pigs were fed a P-depleted diet over a long period of time. In such instances, the need to meet the pig's requirement for growth and tissue accretion would supercede the need to meet the pig's apparent maintenance requirements. In pigs depleted of P for 14 days and then fed diets for 92 days with gradually increasing levels of P, the maximum absorption of P from the diet was observed when P intake levels reached approximate requirement levels, whereas urinary P levels did not exceed estimated inevitable levels until body P reserves were replaced (Rodehutscord et al., 1999). This observation indicates that renal and intestinal homeostasis of P may act independently under different dietary conditions. Whether or not a "true" maintenance P requirement exists for P under normal dietary conditions may be questionable in regards to intestinal and renal releases, yet the estimation of P in endogenously secreted digestive enzymes and fluids can still be considered useful and potentially considerable when diets containing specific feed ingredients are fed and when dry matter intakes are altered (Fan et al., 2001).

Numerous studies have been conducted recently to estimate the endogenous contribution of fecal P attributable to specific feed ingredients fed to pigs, namely corn (Shen et al., 2002) and soybean meal (Fan et al., 2001; Ajakaiye et al., 2003). In each of these studies a regression method was employed, which is dictated by the principle that increasing digestible P intake is linearly related to increasing

P absorption (Fan et al., 2001). In soybean meal, fecal endogenous P outputs were estimated to be on average across treatments 0.25 g/kg dry matter intake for 5 to 20 kg pigs (Fan et al., 2001) and 0.45 g/kg dry matter intake for 20 to 50 kg pigs (Ajakaiye et al., 2003). For corn, endogenous P excretion was determined to be 0.67 g/kg dry matter intake for 20 to 45 kg pigs (Shen et al., 2002). These estimates are unique in that they are the first attempts at associating endogenous P with specific feed ingredients, and they provide evidence that endogenous P excretion may not fluctuate with increasing P intake when fed at levels below the requirement. Further support for this is provided in the lack of correlation between P intake and endogenous P excretion when radio-labeled P was used to measure bone turnover in growing pigs (Fernandez, 1995).

A series of studies were conducted to estimate endogenous P loss reflective of the pure (uninfluenced by dietary ingredients) maintenance P requirement of growing and finishing pigs (Pettey et al., 2004a). By feeding three semi-purified diets formulated at and below the estimated P requirement, a linear relationship of P absorption to P intake was observed and defined by a linear equation for 27, 59, and 98 kg pigs. To account for undigested P collected in the feces, the Y-intercepts from each equation served as an estimate of P excreted in the feces at a theoretical P intake of zero. The estimates obtained for each body weight were constant across P intake levels if the same true P digestibility was assumed for all diets in the study. From the same investigations, urinary P excretion was extremely low and only increased in pigs fed the diet with P levels close to the estimated P requirement. It was determined, therefore, that urinary P is not a source of endogenous P loss from the pig, but rather a response to increased P levels in the diet.

The estimates of fecal endogenous P loss for each weight group were regressed on body weight and the relationship was linear with $Y = 63.056 + 1.632X$ ($R^2 = 0.996$), where X equals body weight (kg) and Y equals endogenous P loss (mg/d). The daily maintenance requirement for P was estimated for every body weight from 20 to 110 kg from this equation. Estimates for given weight ranges are shown in Table 1.

It is evident from the data that although the estimated daily maintenance requirement increases with body weight it is not in a constant proportion to body weight. This observation is in disagreement with previous estimates of the maintenance P requirements of Jongbloed and Everts (1992). These authors estimated the requirement to be 7 mg/kg body weight, which included 1 mg/kg body weight for urinary P loss. The estimate from the current studies may be lower due to the feeding of highly purified diets, which may not have elicited the same secretion of P-containing digestive enzymes or the sloughing of P-containing cells as compared with typical diets fed in commercial situations. It is the contention of this author that the required maintenance P estimates of studies using semi-purified diets better reflects the pure maintenance requirement of the pig. Any influence of dietary ingredients is still yet to be proven and can be addressed by the apparent digestibility estimates of feed ingredients used when estimating total dietary P requirements.

Phosphorus Retention

Phosphorus can be found in every cell throughout the body due to its inclusion in every energetically driven process. The ubiquitous nature of P may seem to make quantification and prediction of whole body P content impossible; yet, the distribution of large pools of P in major tissue groups in the whole body of pigs can be quantified rather easily, and can readily provide a basis for estimating the requirements for P retention. Reference values for the major distribution of P in the body indicate that approximately 75% of whole carcass P is bound in the bone matrix, while the remaining 25% is found in soft tissue stores (Crenshaw, 2001). The latter portion is predominately in the lean muscle mass, mostly due to the greater overall mass of muscle tissue compared with other tissues in the body. In the whole body of the pig, P can also be found in visceral tissue and blood (Just Nielsen, 1972; Mahan and Shields, 1998).

Due to the involvement of P in practically all biological processes, its inherent, structural role in bone formation, and the presence of regulating mechanism in the body to control P homeostasis, the retention of P during the growth period of pigs is the predominant factor dictating net P requirements. Much of the current understanding of P retention in growing and finishing pigs is provided in literature reports from data collected at least 10 years ago, and in some instances up to 30 years ago. The distribution of P in the body of pigs has also been neglected, thus, the understanding of P retention in the body components of pigs, and the relative rate at which P is deposited

in body tissues is poor. This may be the most limiting factor to the development of a comprehensive model describing P requirements for pigs up to this point in time.

The availability of consistent data on the whole body mineral composition of growing pigs is somewhat limited, but numerous studies do exist. It is fair to note that much work has been conducted to study mineral composition of pigs, yet recent investigations utilizing modern pig genotypes are rare. For the purpose of this review, a few select studies were used from the literature representing experiments conducted in the last 20 years. Because the mathematical representation of the data differed between authors, the provided means of P content (g) for each weight group studied were plotted against the average empty body weight (kg) of each group for the purpose of discussion and comparison only. Simple linear relationships were discovered in all data and the slopes of each derived equation were considered to be the amount of P accreted per kg increase in empty body weight. In the investigations of Rymarz et al. (1982), pigs from three different breeds were analyzed and the relationship of P content and empty body weight were: 5.51 (Landrace), 5.42 (Large White), and 4.79 (Hampshire). From this simple comparison it appears that there may be an influence of breed (or body composition differences as defined by breed) on the P retention in growing pigs. Rymarz (1986) conducted another study involving a larger data set of Landrace gilts where the relationship of P accretion to empty body weight gain was 5.0 g/kg. In the most recently published study (Mahan and Shields, 1998), data were collected from Hampshire-Yorkshire-Duroc x Duroc pigs and the mineral content of the whole empty body was determined. The relationship of P content to empty body weight gain was 4.37 in pigs from birth to 145 kg. In the final study reviewed, total mineral content of 36 Large White x Landrace-Large White boars and gilts was evaluated (Hendriks and Moughan, 1993). For boars, 4.18 g of P was gained for every kilogram increase in empty body weight, while the accretion rate in gilts was slightly higher at 4.40 g/kg.

Although comparison between studies is inherently biased, the influence of differing genetics within studies is of interest. One example is the data of Rymarz et al. (1982), where the Hampshire pigs used in the study were fatter at the three heaviest weight groups studied in comparison to both the Landrace and Large White pigs and they also had the lowest P accretion in relation to empty body weight gain. The influence of lean growth changes on P requirements has been discussed in other reports. The relationship of P accretion to protein gain was estimated to be 35 g of P for every kg increase in whole body protein (Jongbloed, 1987). Bertram et al. (1995) showed that pigs with a capacity for moderate or high lean growth had greater lean muscle deposition when increasing available P levels were fed, yet body growth was maximized at a lower dietary intake of available P (0.22% vs. 0.32%) for pigs from a high lean growth genotype. When pigs are treated with pST, lean growth rate is increased and the estimated requirement for P based on lean tissue accretion is greater than untreated pigs (Carter and Cromwell, 1998). Further studies on P accretion in genetically high lean growth pigs, and not pigs whose growth is altered by exogenous hormones, will provide a clearer understanding of the influence lean growth has on P retention.

Two studies were recently conducted to quantify the content of P and rate of accretion in various body tissues of pigs across the growth curve from 18 to 109 kg body weight (Pettey et al., 2004b). All whole empty body (WEB) components were collected (hair, hooves, blood, viscera, head, and carcass). The carcass was split evenly with the left half being ground for analysis while the right half was physically separated into soft tissue, bone, and skin. The estimation of P retained in WEB began by first relating WEB P content (g) to live weight (kg). All pigs from the slaughter investigations (n = 50) were included in the calculation of regression equations. This relationship was linear and is described as $Y = -5.8881 + 4.4115X$; $R^2 = 0.99$. To support the use of a linear equation to describe P gain, the natural logarithm was calculated for live weight and P content, and the linear equation describing the relationship was determined to be $Y = 1.418 + 1.009X$; $R^2 = 0.99$. The slope of the line indicates near unity in the growth of live weight and P mass in pigs from 18 to 109 kg. Therefore, the previous linear equation was used to estimate the P composition of all pigs at their initial and final weights in each weight range. Weight ranges for accretion rate calculations were 18 to 36 kg, 36 to 54 kg, 54 to 73 kg, 73 to 91 kg, and 91 to 109 kg body weight. The five average accretion rates were plotted against live body weight and a quadratic equation was fitted to the data points. The equation describing P accretion (g/d) in growing and finishing pigs from 18 to 109 kg is shown in Figure 1. From

this equation, the accretion rate of P can be estimated for every weight from 20 to 110 kg, and it is assumed that these estimates of P accretion represent the P requirement for tissue growth in growing and finishing pigs.

One primary assumption in the use of the previously described model to estimate P retention in growing and finishing pigs, is that P mass in the body increases linearly to increases in empty body weight. Although strong coefficients of determination can be derived (>0.98) from these relationships, this method also assumes that the P concentration of empty body weight gain is constant across the weight range of a growing pig. This in fact may be true, but should not necessarily be presumed. For these reasons a second model was developed to estimate P retention, utilizing the physically separated tissues and their respective chemical analyses. This allowed for separate estimations of P retention rates in bone, fat-free soft tissue, and the combined empty viscera-head-blood-skin (VHBS) component of the body. With this model, linearity in P content of bone, soft tissue, or VHBS is not assumed.

To begin, the rate of live body weight gain was estimated for the weight ranges described in the first model by calculating weight gain and dividing by the days for each pig within a group to grow from the initial weight in the range to their final slaughter weight. A quadratic equation describing changes in growth rate with increasing body weight was used to calculate a growth rate for every live weight from 20 to 110 kg body weight. For each WEB component (bone, fat-free soft tissue, and VHBS), the percentage of live weight was regressed against live weight and the quadratic equation was used to estimate the corresponding percentage for every body weight from 20 to 110 kg. The percentage P in bone increased linearly with increasing body weight therefore, the linear equation was used to predict percentage P for each body weight. To calculate bone P retention (g/d) the percentage bone of live weight and the percentage bone P at each live weight was multiplied by the rate of body weight gain. The same procedure was used for each other body component, except that fat-free soft tissue remained a constant 45% of live weight from 18 to 109 kg, and P concentration of fat-free soft tissue was also constant at 0.2%, so equations were not needed to estimate these parameters for all body weights from 20 to 110 kg.

One distinct advantage of a partitioned model is the ability to calculate P retention for pigs that vary in their percentage fat-free soft tissue of live body weight. Model 1 only allows for the relationship of lean tissue to P accretion to be established from the data, but does not allow for changes in lean tissue accretion to alter the prediction of P retention. To illustrate the use of Model 2 to estimate P retention in pigs of varying fat-free soft tissue composition, the percentage fat-free soft tissue of live weight was decreased and increased by 5% to 40 and 50%, respectively. Comparisons of the daily P retention rates for these varying fat-free soft tissue percentages are shown in Table 2. The retention of P can also be expressed as a percentage of daily feed intake. These comparisons are also shown in Table 2.

This extrapolation of the data appears to indicate that pigs of varying percentages of whole body lean can have differing dietary requirements for retainable P. One primary assumption from this model is that bone tissue accretion, and thus, bone P accretion, remains constant when lean tissue accretion increases. Outwardly, this contradicts the long-standing notion (Wolff's Law) that increased bone mass accompanies increased muscle mass. Very little is currently understood concerning the relationships of bone mass and lean tissue mass in growing and finishing pigs. Quiniou and Noblet (1995) studied body composition in pigs from different breeds and genotypes and, from their report, dissectible muscle mass increased in Pietrain and synthetic line male pigs compared with Large White barrows and gilts, yet bone mass varied little between pig types. These observations do not hold true, however, when low-lean Meishan pigs are evaluated (Bark et al., 1992; Quiniou and Noblet, 1995). Changes in bone mass when muscle mass, or percentage lean, is increased has also not been shown by dietary manipulations with chromium (Mooney and Cromwell, 1995) or ractopamine (Crome et al., 1996). At this time, based on lack of evidence, we are unable to correct bone P accretion when using a model to estimate Ca and P requirements in varying lean growth genotypes. The growth and degree of mineralization in the skeleton of growing pigs has long been ignored in the comparison of different pig genotypes. Compared with the slight variation in retainable P estimates when fat-free soft tissue is altered, the influence of bone development changes would be much more dramatic on the overall P requirement for a given genotype of pig. Discovering accurate and repeatable means by which skeletal mass can be evaluated in pigs of varying genotypes would greatly

enhance the ability of nutritionists to better estimate Ca and P requirements for tissue growth.

Estimation of Calcium Retention

Although the primary focus of these models concerns the estimation of retainable P in WEB, the relatively static relationship of P and calcium (Ca) in WEB tissues is undeniable. Using the methodology as described for the estimation of P retention in Model 2, the retention rate of Ca can be calculated to provide an estimate of the retainable Ca requirement in whole body tissues. The same equations relating the percentages of WEB components to live body weight were used for the Ca model, with the percentages of Ca in those WEB components replacing those of P. Calcium retention rate peaked at 82 kg body weight (7.04 g/d) and is described by a quadratic equation (Figure 2). When the daily retention of Ca is divided by the observed daily feed intake for each body weight, a dietary retainable Ca (%) requirement for WEB can be determined. Values of 0.30, 0.26, 0.24, 0.23, and 0.21% were estimated for 27, 45, 64, 82, and 100 kg pigs, respectively.

Based on a Ca balance study conducted with 59 kg pigs fed highly digestible calcium carbonate in semi-purified diets (Pettey et al., 2004a), the average Ca digestibility coefficient for pigs consuming adequate dietary Ca levels was 52% (data not shown). Using this value, the estimated total dietary Ca requirement as estimated by this model would be 0.54, 0.51, 0.47, 0.45, and 0.41%, which averaged 93% of the current Ca requirement estimates for each body weight (NRC, 1998).

Net Phosphorus Requirement for Growth

To meet its daily P requirement, a growing pig must consume enough P to account for the endogenous loss of P from the body, and then enough P to meet the retention requirements of growing whole body tissues. It is assumed that the net P requirement would be the sum of these two metabolic requirements. From the data presented thus far a net P requirement can be calculated by simply adding the estimate for maintenance P to the estimate for whole body P retention for each body weight from 20 to 110 kg. These weights were chosen to be the range of net P estimation for this model based on the range of pigs studied in the supporting investigations. The calculated net P requirement for pigs from 20 to 110 kg is shown in Table 3. Daily requirements as estimated using Model 2, and the daily P requirement estimate of the NRC (1998) are shown for comparison purposes. (Figure 3)

Jongbloed and Everts (1992) predicted net P requirements utilizing a factorial approach similar to these methods. These authors estimated P retention to be approximately 5.1 g/kg live weight gain for 30 kg pigs which then decreased linearly (approximately 0.10 g/kg live weight gain per unit increase in live weight) to 4.60 g/kg live weight gain in 110 kg pigs. In comparison, the current model estimates P retention to stay relatively constant in relation to live weight gain. Pigs at 27 and 100 kg had P retention estimates of 4.41 and 4.41 g/kg live weight gain, respectively with an average of 4.40 across all weight ranges. Differences in growth rates, body composition, and feeding strategies can all potentially account for the difference in P retention estimates.

Conclusion

As nutrient management, particularly regarding P, becomes more important to commercial swine operations, there will be a continuing need to better understand the pigs' requirement during the growing-finishing period. The development of models that quantify the various factors influencing P requirements will allow for the flexibility to develop different diets that meet P requirement estimates for pigs of differing genotypes and growth rates. The continuing efforts to estimate P retention in body tissues of larger groups of pigs will only serve to advance the ability of nutritionists to apply these models to commercial swine operations.

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Table 1. Estimates of maintenance P requirements at various weight intervals from 20 to 110 kg body weight

	Body weight range, kg					
	20-35	35-50	50-65	65-80	80-95	95-110
	------- Average weight in range, kg -------					
	27	42	57	72	87	102
Maintenance P requirement, mg/d[a]	107.4	132.0	156.5	181.0	205.5	230.1

[a]Estimated by the linear relationship of endogenous P excretion at zero P intake on body weight: $Y = 63.056 + 1.632X$, $R^2 = 0.996$ (Pettey et al., 2004a).

Table 2. Comparison of varying fat-free soft tissue percentages on estimated daily P retention, and as a percentage of daily feed intake in growing and finishing pigs

	Mean body weight, kg				
	27	45	64	82	100
P retention, g/d					
Lean – 45%	3.06	4.17	4.79	4.87	4.48
Lean – 40%	2.99	4.08	4.68	4.75	4.37
Lean – 50%	3.13	4.27	4.90	4.98	4.58
P retention, % of daily feed intake[a]					
Lean – 45%	0.22	0.19	0.17	0.16	0.14
Lean – 40%	0.22	0.19	0.17	0.15	0.14
Lean – 50%	0.23	0.20	0.18	0.16	0.14

[a]Assumes feed intake does not change with varying fat-free soft tissue content.

Table 3. Net P requirement estimates for 20 to 110 kg growing-finishing pigs

	Live weight range, kg					
	20 - 35	35 - 50	50 - 65	65 - 80	80 - 95	95 - 110
	------- Average weight in range, kg -------					
	27	42	57	72	87	102
Net P required, g/d						
Model[a]	3.16	4.16	4.78	5.06	5.01	4.63
NRC, 1998[b]	3.95	4.50	4.82	4.97	5.02	4.98

[a] Estimated from the data of Pettey et al., 2004a,b.

[b]Available P as estimated by the NRC (1998).

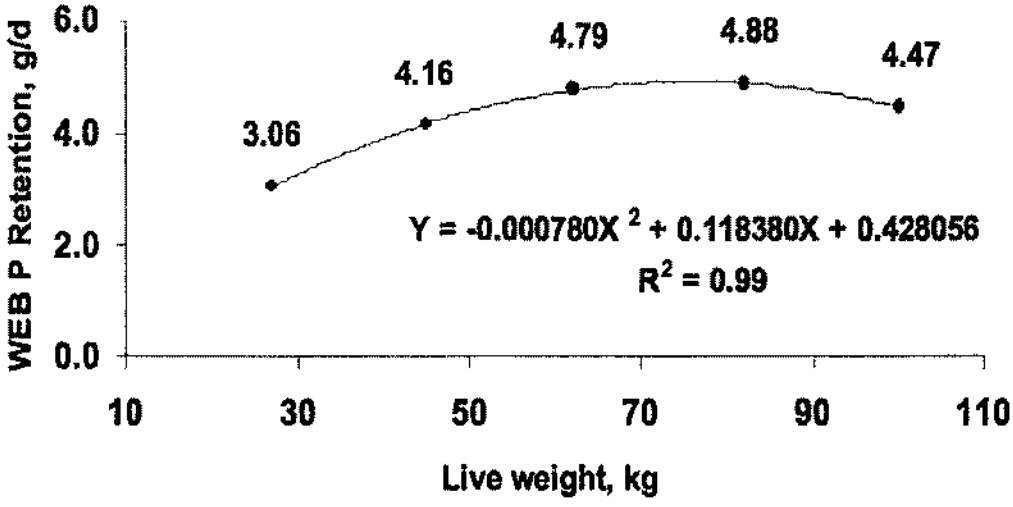

Figure 1. Estimates of whole empty body (WEB) phosphorus (P) retention in growing-finishing pigs from 20 to 110 kg BW (Pettey et al., 2004b).

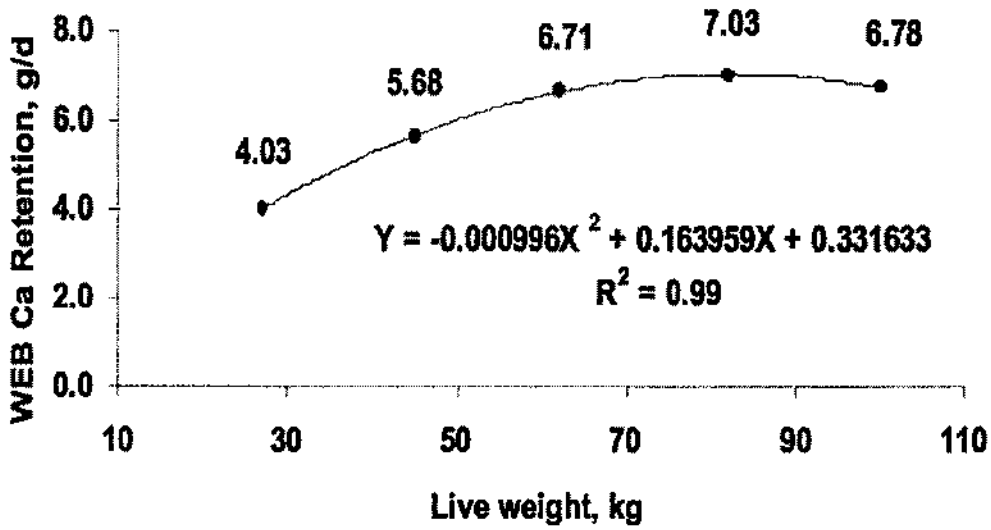

Figure 2. Estimates of whole empty body (WEB) calcium (Ca) retention in growing-finishing pigs from 20 to 110 kg BW (Pettey et al., 2004b).

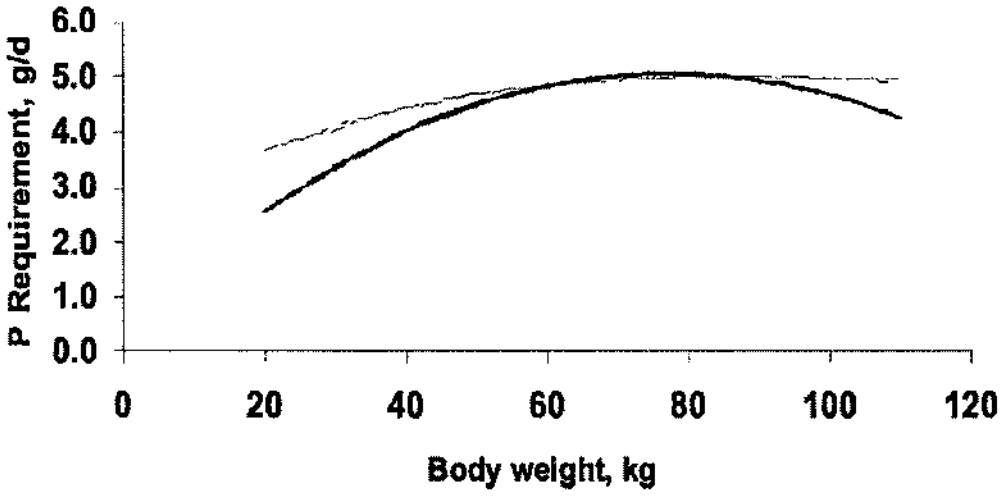

Figure 3. Comparison of P requirement estimates (g/d) of growing-finishing pigs from 20 to 110 kg BW using empirical data (NRC, 1998; solid line) and using a modeling approach (Pettey et al., 2004; dashed line).

Host and Intestinal Microbiota Negotiations in the Context of Animal Growth Efficiency

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Introduction

All major groups of microbes are represented in the gastrointestinal microbiota of mammals although bacteria predominate (Savage, 1977; Mackie et al., 1999). Importantly, bacterial cells outnumber animal (host) cells by a factor of ten and have a profound influence on immunological, nutritional, physiological and protective processes in the host animal (Berg & Savage, 1972; Berg, 1996).

The intestinal microbiota of the pig, and mammals in general, is viewed typically as being beneficial for the host. Indigenous bacteria provide the host with nutrients, including short-chain fatty acids, vitamin K, B vitamins, and amino acids (Savage, 1986; Wostmann, 1996). Intestinal bacteria also prevent colonization by pathogenic organisms by competing more successfully for nutrients or for epithelial attachment sites (Rolfe, 1997). Further, the production by intestinal bacteria of antimicrobial compounds, volatile fatty acids, and chemically modified bile acids, creates a local environment that is generally unfavorable for the growth of enteric pathogens (Rolfe, 1997). While it is certain that commensal bacteria provide both nutritional and defensive functions to the host animal, it is also clear that the host invests substantially in defensive efforts to first sequester intestinal microbes away from the epithelial surface (pathogens and nonpathogens alike), and second to quickly mount inflammatory and immune responses against organisms that manage to breech epithelial defenses.

This commensal relationship has been selected over evolutionary time resulting in a stable microbiota in mature animals that is generally similar in composition and function in a diverse range of animal species. Despite evolutionary stability, the intestinal microbiota develops in individual animals in a characteristic successional pattern that requires substantial adaptation by the host during early life periods. The impact of the developing microbiota as well as the metabolic activities of climax communities require especial consideration when viewed in the context of animal production in which efficiency of growth is a primary objective.

Much of our knowledge regarding the contributions of intestinal bacteria to the development and functions of the mucosal immune system is derived from studies with germfree (GF) animals. These data illustrate the protective nature of commensal bacteria, as well as their role in the development of the mucosal immune system of the host. A brief review is given of 1) the structure and function of mucosal defense mechanisms, 2) studies with GF animals, which support the concept that the mucosal immune system evolved largely in response to the normal microbiota, and 3) the concept of an optimal intestinal microbiota in terms of animal health versus growth performance.

Mucosal defense mechanisms: Structure and function

The mucus biofilm. Intestinal defense functions are organized in a stratified manner, beginning with

the mucus layer and its associated microbiota. The mucus gel layer is an integral structural component of the mucosal surface, acting as a medium for protection, lubrication, and transport between luminal contents and the epithelial lining. Mucus is a heterogeneous mixture of secretions comprised of approximately 95% water and containing electrolytes (Na^+, K^+, Mg^{2+}, and Ca^{2+}), carbohydrates, proteins, amino acids, and lipids (Verdugo, 1990). The viscoelastic, polymer-like properties of mucus are derived from the major gel-forming glycoprotein components called mucins (Forstner et al., 1995). Mucins consist of a peptide backbone containing alternating glycosylated and nonglycosylated domains, with glycosylated regions comprising 70 to 80% of the polymer. The oligosaccharide side chains are linked to the peptide core by O-glycosidic bonds between threonine or serine on the peptide core and N-acetylgalactosamine on the sugar chains (Neutra & Forstner, 1987). N-acetylglucosamine, N-acetylgalactosamine, galactose, and fucose are the four main mucin oligosaccharides (Neutra and Forstner, 1987). Terminal sulfate or sialic acid groups are often added to the oligosaccharide chains, which accounts for the polyanionic nature of mucins at neutral or near neutral pH (Neutra & Forstner, 1987). Secretory mucins in the small and large intestine are synthesized and secreted by specialized columnar epithelial cells referred to as goblet cells, according to their characteristic "goblet" shape (Neutra & Forstner, 1987).

The complexity and especially the extent of the mucus layer is just beginning to be revealed through the development of histological techniques allowing its preservation (Matsuo et al., 1997). Ontogenic changes in the composition of the mucus layer that follow successional changes in the intestinal microbiota and the acquisition of immune functions are consistent with mucus-secreting goblet cells playing a crucial role in intestinal homeostasis (Neutra & Forstner, 1987; Deplancke & Gaskins, 2001). Changes in either the number of goblet cells or the chemical composition of intestinal mucus are detected consistently in response to diverse luminal insults (Neutra et al., 1982; Olubuyide et al., 1984; Dunsford et al., 1991; Sharma & Schumacher, 1995). We have observed an increase in goblet cell numbers in the small intestine of neonatal piglets fed parenterally from birth, as well as an increase in sulfomucin-secreting goblet cells within ileal crypts of parenterally-fed animals (Gannesunker et al., 1999; Conour et al., 2002). Changes in goblet cell populations in the total parenteral nutrition piglet model correlated with altered microbial population profiles, enhanced inflammation, and decreased integrity of the mucosal barrier in response to the lack of enteral nutrition (Ganessunker et al., 1999; Conour et al., 2002; Deplancke et al., 2002). These and emerging data from others indicate that mucus should be considered a dynamic component of intestinal defense, rather than simply a static and viscous barrier that imparts physical protection. The regulatory networks that interface with mucus-secreting goblet cells must be discovered for full adoption of this model.

Protection of the epithelium against intestinal microbes lies in the capacity of mucin carbohydrates to either bind to or repel microbial receptors (Neutra & Forstner, 1987). Intestinal bacteria unable to bind mucus are either excreted via peristalsis and defecation or have evolved growth characteristics that enable their survival in mixed luminal contents. There is a general belief that mucus-resident bacteria prevent the attachment of pathogenic organisms by occupying competitive binding sites. While supporting in vitro evidence exists, much additional in situ data are needed to verify or refute this idea. For example, there is little information on the taxonomy, or the temporal or spatial distribution of bacterial groups that preferentially reside within intestinal mucus. This limitation derives from the difficulties associated with the preservation of the mucus layer with conventional tissue fixation protocols and from the inherent biases of cultivation-dependent microbiological techniques (Mackie et al., 1999). An understanding of the extent and nature of those interactions requires identification of the mucus-associated microbiota as well as elucidation of the molecular basis of host-microbe signaling within this matrix.

The intestinal epithelium. The epithelial cell monolayer underlying the mucus layer is organized into two morphologically and functionally distinct compartments, the crypt regions containing stem cells and Paneth cells, and the villi (small intestine) or epithelial cuffs (large intestine) containing one of several terminally-differentiated epithelial cell types (Gaskins, 1997; Hauck et al., 2005). The formation of tight junctions between adjacent epithelial cells in mature epithelia provides a physical barrier to the external environment. Continual desquamation and renewal of the epithelium further limits opportunities for pathogens to colonize epithelial cells (Potten & Loefler, 1990). In addition to these important innate functions, intestinal epithelial cells are endowed with

immunological functions, including antigen presentation via major histocompatibility complex (MHC) molecules, and the ability to synthesize and secrete numerous inflammatory and regulatory cytokines (Gaskins, 1997). Bioactive cytokines produced by epithelial cells, principally those that are also produced by macrophages, relay information on the relative state of intestinal health to intra-epithelial T lymphocytes (IELs) and immune cells in the underlying lamina propria. Intestinal epithelial-derived cytokines also play key roles in the cellular and functional development of the IEL and lamina propria compartments; however, the specific cytokines involved and their modes of action are not well defined.

These findings point to the importance of understanding the extent and nature of bacterial adherence in the intestine, perhaps with a particular focus on the spatial pattern of adherent populations along the gastrointestinal tract. Colonization of the small intestine even with commensal organisms may result in enhanced sensitivity to otherwise inoffensive luminal stimuli. These considerations may be particularly important in terms of animal growth, given the energetic and nitrogenous costs likely associated with intestinal secretory responses. A better understanding of the biochemical basis of bacterial adhesion might also enable the discovery of non-pharmacological means to control colonization, for example, through the use of synthetic or natural epitopes that mimic mucosal attachment sites.

The lamina propria. Diffuse populations of T and B lymphocytes, plasma cells, macrophages, mast cells, eosinophils, and smaller numbers of dendritic cells and neutrophils, as well as biologically-active fibroblasts, reside beneath the epithelial cell monolayer in the lamina propria (Hinterleitner & Powell, 1991; Kagnoff, 1993). The lamina propria is also well vascularized and densely innervated with a rich plexus of enteric nerves, which play a critical role in intestinal motility, also an important innate defense function.

The complex communication networks that exist between epithelial and submucosal cells are best reflected by the concerted inflammatory responses to luminal insults (Gaskins, 1997). For example, in response to bacterial toxins or food antigens, activated lamina propria cells secrete cytokines and bioactive lipids, which collectively increase motility and blood flow to the intestine, while inhibiting absorption and stimulating water and ion secretion by the epithelium. When integrated, these physiological responses culminate in secretory diarrhea, the most common symptom of intestinal inflammation, regardless of the initiating insult (Hinterleitner & Powell, 1991).

Intestinal T lymphocytes. Intestinal T lymphocytes, residing at sites of initial contact with enteric pathogens, are key components of a "front-line" of defense. Intestinal T cell populations include 1) the intraepithelial lymphocytes (IELs) lying between or immediately beneath the epithelial monolayer, 2) lamina propria T cells, which lie between the villi or crypts away from the epithelial layer, and 3) those residing in Peyer's patches (Guy-Grand et al., 1993). In addition to their anatomic location, intestinal T cell subsets are distinguished by their pattern of ontogenic appearance, their site of maturation, their surface expression of cell differentiation molecules (e.g., CD4 vs. CD8), and by T cell receptor subtype (TCR; $\alpha\beta$ or $\gamma\delta$) subtype (Gaskins, 1997).

The effects of commensal bacteria on the development of intestinal T cells appear to vary according to T cell phenotype. Peyer's patches contain T cell areas with CD4 or CD8 cells, which use the $\alpha\beta$ TCR, and these populations are diminished in the GF state along with a generally reduced lymphocyte cellularity (Macpherson et al., 2001). Of the IEL, the number of $\gamma\delta$TCR cells are relatively unaffected by GF conditions, however, $\alpha\beta$TCR-positive cells are reduced in number, and the normal developmental increase in this population does not occur (Guy-Grand et al., 1993; Umesaki et al., 1993; Helgeland et al., 1996; Helgeland et al., 1997).

Secretory IgA. Secretory IgA is the best described immunologic barrier in the mucosa. Secretory IgA is synthesized by B lymphocytes that originate in bone marrow and migrate to Peyer's patches, a collection of organized germinal centers found along the intestine (Mestecky, 1987; Kraehenbuhl & Neutra, 1992; Kagnoff, 1993). Initially, luminal antigens are transported through specialized epithelial cells (membranous or M cells) overlying Peyer's patches into an interfollicular area where they are presented by resident APC to helper T (T_H) cells. The T_H cells, in turn, secrete cytokines that stimulate B lymphocytes to undergo immunoglobulin class-specific switching to an IgA^+ phenotype (Kagnoff, 1993). After exiting Peyer's patches and passing through the systemic circulation, IgA^+ B lymphocytes migrate or "home" to mucosal surfaces, where upon reexposure to the antigen, plasma cells secrete antigen-specific IgA, which is transported back across the epithelium by a specialized receptor (secretory component), and

released onto the mucosal surface (Mostov, 1994). Mucosal IgA antibodies provide protection primarily by preventing the adherence of bacteria or toxins to epithelial cells, a process commonly referred to as immune exclusion (Kraehenbuhl & Neutra, 1992; Kagnoff, 1993; Stokes & Bourne, 1989).

The homing of differentiated IgA^+ B lymphocytes from Peyer's patches to other mucosal sites including the lungs, female reproductive tract, and mammary gland, provides a mechanism whereby exposure to a pathogen at one mucosal site results in widespread immunity at other mucosal surfaces. This concept is often referred to as the "common mucosal immune system" (Mestecky, 1987).

The influence of luminal antigens and particularly the intestinal microbiota on postnatal development of secretory IgA has been highlighted in animal studies demonstrating that both the size and number of Peyer's patches are reduced significantly in germfree pigs (Pabst et al., 1988; Rothkötter & Pabst, 1989; Rothkötter et al., 1991) and rodents. This phenomenon has been studied in more depth in rodents where it has been demonstrated that monoassociation of germfree animals with certain commensal bacteria can restore normal development of the secretory IgA system (Cebra et al., 1980; McCracken & Gaskins, 1999).

Lessons from the 'germfree' state

Characteristics of GF animals. The immunological characteristics of GF animals are easily distinguished from those of conventional (CV) animals harboring a microbiota. For example, most immunologically relevant organs, such as the small intestine and mesenteric lymph nodes, are smaller, weigh less, and exhibit a reduced cellularity in GF animals (Gordon & Wostmann, 1960; Gordon et al. 1966). In addition, lymph nodes, spleen, and other lymphoid tissues have few germinal centers—sites where antigen presentation occurs, leading to B cell proliferation and the differentiation of antibody-secreting plasma cells (Thorbecke, 1959; Gordon & Wostmann, 1960; Pollard, 1967a). Germinal centers, which are present in GF animals are suspected to result from a) basal activity, b) dietary antigens, or c) responsiveness to endogenous viruses (Pollard, 1967a,b). The thymus grows at a slower rate in GF animals and never reaches the size attained in CV mice although it does follow a pattern of growth and later regression similar to that observed in CV animals (Bealmear, 1965, 1980). Thymus atrophy has a direct effect on the development of peripheral T cell populations, as well as an indirect effect on antibody development via the reduced number of T helper cells required for B cell maturation and activation.

Cell-mediated immunity has not been well characterized in GF mice. However, evidence does exist for delayed type hypersensitivity and normal allograft responses in GF mice (MacDonald & Carter, 1978; Bealmear, 1965). Regarding humoral immunity, GF mice have low concentrations of immunoglobulins (Wostmann, 1961; Sell, 1964a,b) and little or no detectable IgA (Asofsky & Hylton, 1967). GF mice do mount antibody responses to antigen, but at lower concentrations than CV mice (Olson & Wostman, 1966a, b). Germfree mice are also significantly more resistant to LPS-induced lethality than CV mice (Kiyono et al. 1980). Transfer of T cells from CV to GF mice lowered this resistance, which led to the hypothesis that Gram negative bacteria in the intestine contribute to the production of a T cell population, which regulates B cell responses to LPS (McGhee et al., 1980).

Substantial differences have been demonstrated between GF and CV animals in terms of the numbers and function of macrophages, which are among the cell types most responsive to microbial alterations. For example, relative to CV animals, macrophages from axenic animals are: 1) fewer in number (Woolverton et al., 1992); 2) less active metabolically (Heise & Myrvik, 1966); 3) less capable of antigen degradation (Bauer et al. 1966); 4) less responsive to chemotactic stimuli (Abrams and Bishop, 1965; Jungi & McGregor, 1978; Morland et al. 1979); 5) exhibit diminished tumoricidal capabilities (Johnson & Balish, 1981); and 6) decreased microbiocidal activity toward certain pathogens (MacDonald & Carter, 1978).

Reassociation of GF animals with commensal bacteria. Upon introduction of a complete repertoire of commensal bacteria, GF animals develop a physiology comparable to that observed in CV animals. Within 2 days, the cecum becomes smaller and thicker-walled, with more solid contents than GF animals; the spleen, lymph nodes, and Peyer's patches develop germinal centers and begin to grow, reaching CV dimensions within a week of association; the lamina propria is thickened and becomes more cellular; and serum immunoglobulin concentrations increase (Olson and Wostmann, 1966b; Carter & Pollard, 1971; Bealmer, 1980). Coliforms, which colonize the host

immediately following exposure, are thought to be responsible for these changes, as strict anaerobes did not colonize until much later (Mayhew & Pollard, 1971). Differential host responses to specific bacterial species led Dubos et al. (1965) to suggest that, among the bacteria normally found in the host intestine, some are truly symbiotic and constitute the indigenous microbiota, while other intestinal microbes gain access to the host due to their presence in the environment but do not establish a symbiotic relationship. Many subsequent studies have shown that the host reaction to individual species varies by microbial species, with some provoking a strong immunological response, and others producing little, if any, noticeable effect (Wagner, 1959; Carter & Pollard, 1971, 1973; Balish et al. 1972; Berg and Savage, 1972; Foo & Lee, 1972; Moreau et al. 1978, Morishita & Mitsuoka, 1973; Wells & Balish, 1980; Lee, 1984; McCracken & Gaskins, 1999).

Using conventional and germfree pigs of different ages, Rothkötter and coworkers (1989, 1991) reported useful data on the influence of age and the intestinal microbiota on the cellular composition of the intestinal immune system. Crude separations of epithelial and lamina propria compartments demonstrated that total yield of immune cells from intestine of young pigs (5 days old) was only ten percent of that obtained from older animals (45 days old), and that intestinal lymphocyte numbers of nine-month-old pigs were about fifty percent lower than those from fourteen month-old animals. Cell yield and lymphocyte subset patterns from the intestine of forty-five-day-old germfree piglets were comparable to those of five-day-old conventional animals. These data further demostrate that the commensal microbiota is a major stimulus for the postnatal development of intestinal immune cell compartments.

Summary and conclusion

Intestinal adaptations mounted in response to the commensal microbiota carry costs that become most apparent when considered in the contemporary context of animal growth efficiency. Specifically, gastrointestinal tissues represent approximately 6% of body weight but are responsible for 10 to 20% of whole body CO_2 production (Stoll et al., 1999), up to 50% of the whole body turnover of some essential amino acids (Stoll et al., 1998; Van Goudoever et al., 2000), and consume approximately 35% of the total protein-N intake of the animal (Reeds et al., 1999). Moreover, only 10% of the total protein synthesized by the GI tract is accumulated as new mass. Although this is often taken to signify a high rate of intracellular proteolysis, it appears from recent work that the difference between mucosal protein turnover and accretion largely reflects protein loss in sloughed epithelial cells or as secreted products such as mucus (Reeds et al., 1993; Fuller & Reeds, 1998).

That bacterial colonization of the intestine negatively impacts the efficiency of animal growth is well documented through data from studies with GF animals, and by increasing evidence that the growth enhancing effects of antibiotics likely reflect their ability to both decrease intestinal bacterial colonization and alter community profiles. For example, oral antibiotics do not induce a growth-response in GF animals (Coates et al., 1963), while colonizing GF animals with normal intestinal bacteria depresses growth (Coates, 1980). Growth depression is thought to result from the increased maintenance costs associated with host responses to the variety of catabolic processes that mediate bacterial growth in the intestine (Gaskins, 2001). However, quantitative data to support or refute that concept are limited.

Host sensitivity to the intestinal microbiota is further suggested by parallel developmental and regional differences in intestinal structure and function and microbial density, as well as the reduction in epithelial cell turnover and intestinal secretory activity in GF animals. Thus, regulatory cues must exist that enable the host to monitor the extent of bacterial colonization and activity. If this is so, then opportunities should exist for improving animal growth efficiency through the manipulation of the dynamic equilibrium between the intestinal microbiota and host defense functions. These issues raise the important point that while there is presumably a microbiota that optimizes intestinal health, the maintenance of this population becomes part of the nutrient requirements of the host and thereby affects whole body growth efficiency.

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The emerging picture of diet effects on gastrointestinal microbial populations

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Summary

Several dietary ingredients and/or additives can reduce the susceptibility of pigs to microbial colonization and subsequent infection, disease and possibly death. These ingredients and/or additives include cereal grains, milk products, spray-dried plasma, fermented feed, organic acids, microminerals, direct-fed microbials, prebiotics and plant extracts. All are reported to have beneficial effects on the gastrointestinal microflora of pigs, with particular emphasis on health and growth promotion of nursery pigs. These effects may occur at both low and high inclusion levels of the various dietary ingredients and/or additives and for varying lengths of inclusion time in the diet. The response of the gastrointestinal microflora can be evaluated both qualitatively and quantitatively by various molecular techniques.

Introduction

Diets are formulated for swine with one goal in mind and that is to enhance the health status and performance of the pig. These dietary ingredients influence not only the growth of the pig but that of the diverse microflora which inhabit the gastrointestinal tract. Similar to the pig, the microflora are affected by alterations in the external environment, which may include new housing, change in form and composition of diet, variation in ambient temperature and introduction to new pen-mates. These changes often lead to physiological stress and hence modifications within the internal environment of the pig which is occupied by distinct niches of microbes. An upset in this delicate ecological balance can lead to a shift toward invasion and subsequent colonization by pathogenic microbes into the tissue and ultimately the systemic system. This may manifest as subclinical digestive disturbances or decreased production performance (Fox, 1988) which can then lead to clinical infection, disease and possibly death of the pig.

In order for both the host and microbes to survive, there needs to be a microbial balance within the gastrointestinal tract. The host benefits by having a small population of microbes in the stomach and small intestine, as there will be more nutrients available for digestion and absorption by the host animal (Jensen, 1998). In contrast, large numbers of microbes in both the cecum and large intestine are beneficial rather than harmful due to the ability of the host to utilize the end products of microbial fermentation of nutrients not hydrolyzed by intestinal enzymes (Jensen, 1998). This microbial balance is favorable as long as the number of pathogenic microbes is kept to a minimum. When the gastrointestinal tract becomes overwhelmed by pathogenic microbes, this may result in increased metabolic needs of the pig to mount an immune response and regenerate intestinal tissue to replace that damaged by the invasive microbes (Gaskins, 2001). By decreasing the numbers of pathogenic microbes, the metabolic needs of the pig is reduced with an increase in available energy and nutrients for absorption resulting in enhanced performance.

According to Jensen (1993), diet composition is probably the single most important factor for microbial activity in the gastrointestinal tract of monogastric animals, with the type and quantity of ingredients serving as substrates for selective microbes. Savage

(1977) claims that any physiological change that would increase or decrease peristalsis, the quantity of secreted gastric HCl or perhaps the amount of mucus secreted anywhere in the gastrointestinal tract, could conceivably alter the microbial communities in local habitats.

Before antimicrobial growth promoters become unavailable for future use, swine nutritionists together with swine producers must focus their attention on the selection of appropriate dietary ingredients and additives. These additives may not be "alternatives" to antimicrobial growth promoters in relation to improved performance, but can be effective in altering the gastrointestinal microbial population. This alteration may be through enhanced growth of beneficial microbes or by reduction/removal of potential pathogens, thus possibly enhancing the health and performance of the pig.

General Dietary Effects

Typical dietary ingredients such as cereals and plant based-meals serve as sources of energy, protein, fat, minerals and vitamins and thus are not selected based on their ability to influence the gastrointestinal microbial population. However, these ingredients do influence the quantity and type of substrates available to the microbes, and thus have direct control over processes of fermentation in the hindgut of the pig (Jensen et al., 2003). The constituents of the diet that are selected for the purpose of affecting the microbial population are those that are considered to be dietary or non-dietary additives. The majority of this review will focus its attention on those additives and their reported effects on the gastrointestinal microflora.

Cereal Grains

In the United States, corn is the predominant cereal grain serving as the energy source in swine diets. In other countries, swine diets include such cereal grains as sorghum, barley, wheat and rice. These grains are widely available and often less expensive in comparison to corn (Hongtrakul et al., 1998).

There are two distinct and chemically well-defined types of plant polysaccharides: storage polysaccharide starch and cell wall polysaccharides or non-starch polysaccharides (Englyst, 1989). Cereal grains generally fall into the category of storage polysaccharide starch. Non-starch polysaccharides are recognized as the principal component of dietary fiber (Trowell et al., 1985). Dietary fiber is defined as plant polysaccharides and lignin which are resistant to hydrolysis by the digestive enzymes of man (Trowell et al., 1976). Feeds that are high in dietary fiber are often avoided in swine diets, due to their low digestibility.

Research has shown that cereal grains have an effect on the gastrointestinal microflora. Drew et al. (2002) compared the effects of feeding either a barley-, corn- or wheat-based diet to weaned pigs on the microbial populations in the ileum and cecum. The barley diet lowered the counts of Enterobacteria in the ileum, while both the barley and wheat diets increased those of *Lactobacillus* spp. compared to corn (Drew et al., 2002). In the cecum, barley diets increased the populations of anaerobes and *Lactobacillus* spp., while both barley and wheat diets increased the counts of *Bifidobacterium* spp. compared to the corn diet (Drew et al., 2002). Compared to barley, wheat decreased the numbers of *Clostridium* spp. and aerobes (Drew et al., 2002). *Lactobacilli* are considered to have a symbiotic relationship with the host pig and *Bifidobacteria* serve as beneficial microbes that ferment non-digestible components of the diet to produce substrates that can be utilized by the pig. Therefore, an increase in their numbers can be beneficial to the pig.

Hill et al (2005) also fed corn-, wheat- or barley-based diets to weaned pigs and measured their effects on the microbes present within the ileum digesta. All three cereals caused a dominance of *Lactobacillus* spp. in the ileum with a majority being identical to *Lactobacillus amylovorous* (Hill et al., 2005). *Clostridium* spp. counts were most abundant in corn (12% of clone libraries vs. 8% for wheat and 2% for barley); *Streptococcus* spp. represented 6% of clone libraries in barley and 1% each for both corn and wheat; *Bacillus* spp. clone libraries were in corn and wheat only but represented a low percentage (Hill et al., 2005). Similar to the study conducted by Drew et al. (2002), this study reported high numbers of *Lactobacillus* spp., which can be seen as beneficial considering that this genus of bacteria is often used as a probiotic or direct-fed microbial to improve the microbial balance and health status of pigs.

The inclusion of dietary fiber in the form of non-starch polysaccharides can influence the microflora of the gastrointestinal tract. Fiber tends to have its largest effect on the microflora of the cecum and large intestine, with the non-starch polysaccharides being important energy substrates for microbial fermen-

tation (Pluske et al., 2001). Hogberg et al. (2004) reported that both ileo-cecal and rectal coliforms and total gut microflora of growing pigs were influenced by dietary cereal non-starch polysaccharide content. In a study conducted by Durmic et al. (1998), they reported that the populations of total anaerobes and *Bacteroides* spp. were increased while the populations of *Clostridium* spp., *Lactobacillus* spp. and *Enterobacteria* were decreased in the colon of pigs fed diets high in resistant starch compared to those fed diets high in non-starch polysaccharides. Reid and Hillman (1999) reported that the addition of dietary fiber reduces the population of coliform bacteria in the large intestine of pigs. Jensen (2001) compared the effects of feeding diets containing low fiber, oat bran or wheat bran and found that the microbial activity was low in the hindgut of pigs fed the low fiber diet. When oat bran was added to the low fiber diet, the microbial activity increased in the ileum and cecum, while the addition of wheat bran increased the microbial activity in the distal region of the hindgut (Jensen, 2001). The reason for the difference was due to the fact that oat bran consists of easily fermentable dietary fiber, while wheat bran consists of more slowly fermentable fiber (Jensen, 2001).

Milk Products

Milk products such as dried whey, dried skim milk and lactose are included in the diet as an additional source of digestible energy. Lactose is converted to lactic acid through microbial fermentation in the stomach which lowers the pH and creates a more acidic environment. Lactic acid is considered to be important for keeping the numbers of *Escherichia coli* low in suckling piglets (Partanen, 2001). With an increase in lactic acid production, there should be an expected increase in the population of lactic-acid producing bacteria.

Krause et al. (1995) reported no effect of lactose on the number of *Lactobacillus* spp. adhering to the wall of the digestive tract or present within the digesta of weanling pigs. In contrast, Wells et al (2005) found an effect on the microbial populations present in the feces of growing-finishing swine fed diets containing dried skim milk (10% of diet). In pigs fed dried skim milk, from 14-18 weeks of age, there was a decrease in the populations of Enterobacteria, coliforms and *Escherichia coli* compared to pigs fed diets without dried skim milk (Wells et al., 2005). *Lactobacillus* spp. populations were lower in pigs not fed dried skim milk while pigs fed dried skim milk maintained consistent counts from the start to conclusion of the experiment (Wells et al., 2005). Although there was no difference between diets, at 22 weeks of age the populations of Enterobacteria, coliforms and *Escherichia coli* were greater than at week 18 for the pigs not fed dried skim milk with less change in these microbial populations seen in pigs fed dried skim milk (Wells et al., 2005). Conclusions can not be drawn at this time, but it appears that milk products can influence the gastrointestinal microflora.

Spray-Dried Plasma

Spray-dried plasma is an animal-derived protein consisting of a complex mixture of plasma fibrinogen, immunoglobulins, albumin and other components (APC, 2000). The feeding of spray-dried plasma may be associated with changes in the intestinal microflora (van Dijk et al., 2001) by preventing the adhesion of pathogenic bacteria to the gastrointestinal mucosa due to the presence of glycoproteins present in spray-dried plasma (Nollett et al., 1999). Coffey and Cromwell (1995) reported that the beneficial effects of spray-dried plasma are more pronounced under production conditions with high pressure of pathogens as compared to environments with optimal hygiene.

The effect of dietary spray-dried plasma in microbial challenge studies has produced mixed results. van Dijk et al. (2002) reported that there were no differences in the counts of inoculated *Escherichia coli* in the jejunum, cecum and rectum of weaned pigs fed either a diet containing 8% spray-dried plasma or soybean meal plus whey powder. In contrast, Torrallordona et al. (2003) challenged weanling pigs with *Escherichia coli* K99 and found that in pigs fed diets containing 7% spray dried plasma in the absence or presence of colistin (anti-microbial), there was an increase of 1.14 log cfu *Lactobacillus* spp./g ileal digesta and an increase of 0.45 log cfu *Lactobacillus* spp./g cecal digesta compared to pigs fed diets not containing spray-dried plasma. However, there was no reported effect of spray-dried plasma on the numbers of *Enterococcus* spp., *Escherichia coli* and *Clostridium perfringens* in both ileal and cecal digesta (Torrallordona et al., 2003). Although spray-dried plasma has been shown to enhance the performance of pigs (van Dijk, 2001), it is an expensive protein source and the concerns regarding the fact that it is an animal-derived protein may result in its dietary inclusion being limited in the future.

Fermented Liquid Feed

The dietary inclusion of fermented liquid feed has gained interest in Denmark and other European countries regarding its possible beneficial effect on the gastrointestinal microbial population of the pig (Hojberg et al., 2003). Fermented liquid feed has been defined as a mixture of feed and water stored in a tank at a certain temperature for a specific period of time before it is fed to the animals (Canibe and Jensen, 2003). Non-fermented liquid feed is defined as a mixture of feed and water made immediately before feeding or in the trough at feeding (Canibe and Jensen, 2003). From the instant the feed and water are mixed, there is a possibility that fermentation will begin (Canibe and Jensen, 2003).

Fermented liquid feed has been reported to have beneficial effects on the gastrointestinal microflora when included in the diet of nursery through finishing pigs. Mikkelsen and Jensen (1997) found that fermented liquid feed decreased the numbers of Enterobacteria along the gastrointestinal tract of pigs compared to pigs fed diets containing either dry feed or non-fermented liquid feed. van der Wolf et al. (2001) reported that fermented liquid feed decreased *Salmonella* seroprevalence in pigs. However, the feeding of liquid compound feed soaked in a trough for a few hours was shown to increase the risk of *Salmonella* infection in pigs (van der Wolf et al., 1999). Canibe and Jensen (2003) found that fermented liquid feed significantly lowered the counts of total anaerobes in the stomach and mid-colon; lowered Enterobacteria counts along the entire gastrointestinal tract and increased the counts of lactic acid bacteria in the stomach and small intestine of growing pigs compared to pigs fed a diet containing non-fermented liquid feed or dry meal feed. They also reported a constant lower pH and higher concentration of organic acids in the stomach of pigs fed fermented liquid feed compared to a fluctuating gastric pH in pigs fed the other two diets.

These effects of fermented liquid feed on the gastric environment can impair microbial metabolism of pathogenic microbes that prefer a more neutral pH and thus be beneficial to the pig (Canibe and Jensen, 2003). Hojberg et al (2001) fed the same fermented liquid feed and dry meal feed diets as Canibe and Jensen (2003) and measured lower fermentation capacity in both the cecum and colon of pigs fed fermented liquid feed, which correlated with lower microbial counts in these two segments.

The antimicrobial effects may be due to the composition of fermented liquid feed. Fermented liquid feed contains high concentrations of lactic acid, volatile fatty acids and large numbers of *Lactobacillus spp.* (van Winsen et al., 2001). Thus, fermented liquid feed can improve gastrointestinal health by lowering gastric pH values to 4 or below (Jensen and Mikkelsen, 1998) due to the production of high levels of gastric acid (Jensen et al., 2003). The increase in lactic acid is accompanied by an increase in the populations of lactic acid bacteria in the proximal portion compared to a decrease in the distal portion of the gastrointestinal tract, as was reported in growing pigs fed fermented liquid feed (Jensen and Mikkelsen, 1998). The decrease was likely due to a lower amount of substrate available to the microbes in the distal portion since fermented liquid feed has a lower carbohydrate content than dry meal feed (Jensen and Mikkelsen, 1998). It appears that fermented liquid feeding may be a viable option, if the pigs will consume feed that may have an unfavorable taste due to fermentation.

Organic Acids

The inclusion of organic acids and their salts in the diets of nursery pigs has been more prevalent in Europe compared to the United States. This is largely due to the combination of both negative and positive effects of organic acids. High dietary levels of organic acids are corrosive to cement and galvanized steel used in swine housing (Best, 2000) and they can cause decreased palatability and a subsequent increase in feed refusal (Partanen and Mroz, 1999). However, organic acids reduce the pH of digesta, which creates less optimal conditions for microbial growth and enables the acids to enter into microbial cytoplasm and disrupt the life cycle of the microbe (Mateos et al., 2001; Roth and Kirchgessner, 1998). By maintaining the gastric pH below 4, Enterobacteria can be killed (Knarreborg et al., 2002). The antimicrobial properties of the cations and anions of organic acids and their salts are due to the dissociation of the acids after passing through the bacterial cell wall which leads to a decrease in the internal bacterial pH and a collapse of the protein motive force, resulting in impairment of bacterial cell metabolism and/or death (Partanen and Mroz, 1999; Russell et al., 1998).

In an *in vitro* study conducted by Knarreborg et al. (2002), the efficiency of organic acids in be-

ing able to cause a decline in viability of coliform bacteria was in increasing order: propionic<formic< butyric<lactic<fumaric <benzoic. They also demonstrated that the antibacterial effect is pH rather than concentration dependent as at the same concentration of acid, as the pH decreased, the antibacterial effect increased (Knarreborg et al., 2002).

Citric Acid

Citric acid is utilized either alone or in combination with another organic acid in the diet. There is limited data available on its use in pig diets. Scipioni et al. (1978) reported a decrease in total anaerobes and *Escherichia coli* in weanling pigs fed citric acid. In contrast, Risley et al. (1992) found that 1.5% citric acid in the diet had no effect on total anaerobes, *Lactobacillus* spp., *Clostridium* spp., and *Escherichia coli* in the stomach, jejunum, cecum or lower colon. Following an *Escherichia coli* challenge, the addition of 1.5% citric acid to the diet of weanling pigs had no effect on the severity or incidence of diarrhea and no effect on the numbers of *Lactobacillus* spp. or *Escherichia coli* along the gastrointestinal tract (Risley et al., 1993).

Lactic Acid

Similar to other dietary acids, lactic acid has had varied effects on the gastrointestinal environment of pigs. Lactic acid has been shown to be consistent in reducing gastric pH and coliform populations in pigs (Jensen, 1998). Ratcliffe et al. (1986) supplemented milk with 1% lactic acid and found lower counts of coliforms and *Lactobacillus* spp. in the stomach and duodenum of 2 week old weaned pigs with no effect on the number of coliforms in the colon. Cole et al. (1968) found that the addition of 0.8% lactic acid to the diet reduced the levels of *Escherichia coli* in both the duodenum and jejunum of 8 week old piglets. In nursery pigs fed diets containing 0.75% pure lactic acid, there was a significant reduction in total anaerobes in the ileum and lower pH values along the gastrointestinal tract at day 7 post-wean compared to pigs fed the lactose and negative control diets (Palacios, 2004). Maribo et al. (2000) found that lactic acid but not formic acid stimulated the growth of yeast in piglets but was unable to determine if the yeasts play a detrimental role in the gastrointestinal tract.

Formic Acid

Since formic acid is not approved for use in the United States' swine industry, the salt form of this acid is incorporated into the diet of weanling pigs. Both forms have been shown to have an effect on the gastrointestinal microbial population of pigs.

Dietary inclusion of 1.2% formic acid lowered the counts of *Escherichia coli* (Bolduan et al., 1988) and coliform bacteria in the gastrointestinal tract as well as a reduction in the incidence and severity of diarrhea in piglets post-wean (Eckel et al., 1992; Tsiloyiannis et al., 2001). Gedek et al. (1992) reported that 1.8% formic acid resulted in higher *Escherichia coli* counts in duodenal digesta, lower *Escherichia coli* counts in cecum and large intestine and lower counts of both *Lactobacillus* spp. and *Bifidobacterium* spp. in the small and large intestine of piglets. In piglets fed 1.25% formic acid, there was a decrease in the numbers of *Escherichia coli* and *Bacteroides* spp. in the duodenum and jejunum and lower Eubacteria counts in the jejunum with no effects on *Lactobacillus* spp. counts in the gastrointestinal tract (Kirchgessner et al., 1992). Gabert et al. (1995) reported that 1% formic acid had no effect on the microbial populations with the most predominant group being the gram positive anaerobes in pigs fed diets with or without formic acid in the presence of fish meal or high levels of both calcium and phosphorus.

Potassium Diformate

In both *in vivo* (1.2-1.8% of diet) and *in vitro* studies, potassium diformate, the salt form of formic acid, has been shown to have a significant antimicrobial effect on coliform growth (Overland et al., 2000; Canibe et al., 2001; Knarreborg et al., 2002; Mellor, 2003). When potassium diformate is diluted in the gastrointestinal contents of a pig, it dissociates into formic acid, formate and potassium (Mellor, 2003). When the pH is low in the intestinal tract, the undissociated formic acid can penetrate the bacterial cell wall and perhaps cause death of the bacteria (Mellor, 2003).

Diets supplemented with 0.9% or 1.8% potassium diformate reduced the number of coliforms and *Streptococcus* spp. in the stomach and coliforms in the colon, with no effect on *Lactobacillus* spp. in the gastrointestinal tract of weaned piglets (Fevrier et al., 2001). The addition of 1.8% potassium diformate to the diet of weaned pigs had a tendency to lower total fecal anaerobic bacteria counts, throughout the

28 day experiment compared to negative control pigs (Canibe et al., 2001). On different sampling days in the same experiment, there were lower counts of lactic acid bacteria and coliforms in the feces (Canibe et al., 2001). At 7 days post-wean, there were significantly lower counts of total anaerobic bacteria and lactic acid bacteria with no difference in coliform counts along the entire gastrointestinal tract of pigs fed potassium diformate (Canibe et al., 2001). At 29 days post-wean, potassium diformate significantly lowered the counts of total anaerobic bacteria in the stomach and ileum and counts of lactic acid bacteria with numerically lower counts of coliforms along the gastrointestinal tract (Canibe et al., 2001). Overland et al. (2000) supplemented the diets of grow-finish pigs with either 0.6% or 1.2% potassium diformate and found lower coliform (*Escherichia coli, Salmonella* spp., *Shigella* spp. and *Enterobacter* spp.) counts in the digesta of the duodenum, jejunum and rectum compared to pigs fed the diet without potassium diformate.

Trace Minerals

Zinc and copper are the main microminerals that have been reported to have effects on gastrointestinal microflora. They are believed to have a bacteriostatic and/or bactericidal effect (Jensen et al., 2003).

Zinc

High levels of supplemental zinc to the diet of nursery pigs, has been shown to enhance performance. The improvement in performance may be due to altered microbial activity within the gastrointestinal tract. Gram positive bacteria are more susceptible to zinc oxide than are gram negative bacteria and *Streptococcus* spp. (Mavromichalis et al., 2000). Also, it has been suggested that high dietary levels of zinc oxide may help control *Escherichia coli* scours (Poulsen, 1989), which can improve gut morphology (Carlson et al., 1999). Improved fecal consistency scores have been reported with 3000 ppm zinc (zinc oxide) compared to 150 ppm zinc (zinc oxide) (McCully et al., 1995).

Li et al. (2001) reported no effect of zinc oxide on the microbial populations of ileal digesta and feces in newly weaned piglets. In addition, Jensen-Waern et al. (1998) found that 2500 ppm zinc (zinc oxide) had no effect on the numbers of fecal *Escherichia coli* and *Enterococcus* spp. in weaned pigs. The populations of lactic acid bacteria were reduced in all segments of the gastrointestinal tract of pigs fed diets containing 2500 ppm zinc (Jensen et al., 2003). These results agree with claims that gram positive bacteria, such as *Lactobacillus* spp., are more sensitive to high dietary concentrations of zinc oxide compared to gram negative bacteria that include coliforms.

Copper

Copper may alter the microbial populations within the pig's gastrointestinal tract (Bunch et al., 1961). Growth rate and feed efficiency responses in nursery pigs to high dietary copper (200-250 ppm) are similar in magnitude to responses from antimicrobials (Cromwell, 2001). This gives an indication that copper may have a similar mode of action to antimicrobials. In the comparison of the effect of high dietary copper on growth performance, Shurson et al. (1990) reported a positive response on daily growth rate and feed efficiency in conventional pigs and a negative response in germ-free pigs, but suggested this may have been a systemic rather than an antimicrobial effect in the gastrointestinal tract.

The effects of copper on gastrointestinal microbial populations have been mixed. Bunch et al. (1961) reported that copper sulfate significantly reduced the number of *Lactobacillus* spp., total aerobic and anaerobic microbes when fed to piglets for 6 weeks, while copper oxide only significantly lowered the numbers of coliforms. Varel et al. (1987) found that copper sulfate reduced the number of *Streptococcus* spp. in the gastrointestinal tract of pigs. High dietary concentrations of copper reduced numbers of lactic acid bacteria and increased coliform counts in the gastrointestinal tract of pigs (Jensen, 1998). In contrast, 250 ppm copper sulfate had no effect on the populations of *Lactobacillus* spp., *Streptococcus* spp., *Escherichia coli, Clostridium perfringens, Bacteroides* spp. and yeasts in the gastrointestinal tract of five month old pigs (Smith and Jones, 1963).

Direct-Fed Microbials

A direct-fed microbial or probiotic is defined as a live microbial food supplement that beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989). There are three defined categories of direct-fed microbials. These include: 1) lactic acid bacteria which occur naturally

in the digestive tract; 2) bacteria belonging to the genus *Bacillus* that grow on soil; and 3) *Saccharomyces* yeasts which normally grow on plant material (Simon et al., 2003). The selection criteria for direct-fed microbials include the following: 1) must be classified as GRAS (Gibson and Fuller, 2000); 2) survival and establishment of the fed microbial after ingestion (Gibson, 1999); 3) resistance to low stomach pH and bile (Gibson and Fuller, 2000); 4) ability to adhere to gut epithelium (Gibson and Fuller, 2000); and 5) inhibitory effect on pathogens through production of acid or by competitive exclusion (Gibson and Fuller, 2000).

The effects of some direct-fed microbials are dose-dependent, with some only being effective during the inoculation period after which they disappear within a few days post-inoculation (Sakata et al., 2003). A possible reason for their short-term effect could be the failure of some direct-fed microbials to adhere to gut epithelial tissue of young pigs, which may result in their removal from the gastrointestinal tract due to peristalsis of the contents (Krause et al., 1997).

Bacteria

Lactobacilli establish in the pig's gastrointestinal tract shortly after birth and remain a predominant part of the microbial community throughout the pig's life (Naito et al., 1995). *Lactobacilli* are important inhabitants of the gastrointestinal tract due to their possible antagonistic activities toward other bacteria (Henriksson et al., 1995).

The dietary inclusion of various strains of *Lactobacilli* have had varied effects on the gastrointestinal microbial population of pigs. Nursery pigs fed milk supplemented with *Lactobacillus brevis* had higher coliform counts in the duodenum on day 10 post-wean and lower counts on day 20 post-wean compared to pigs not receiving this direct-fed microbial (Davis et al., 2005). In the same experiment, pigs fed the direct-fed microbial had lower *Escherichia coli* counts in the jejunum on day 10 post-wean but no effect on the day 20 or 38 post-wean counts compared to pigs not receiving the direct-fed microbial (Davis et al., 2005). Muralihadra et al. (1977) reported that *Lactobacillus lactis* reduced the fecal coliform and *Escherichia coli* counts and increased the *Lactobacillus* spp.counts in the proximal and distal small intestine mucosa of piglets compared to those fed a negative control diet. Santos et al. (2003) found no significant effect of supplemental *Lactobacillus* spp. on intestinal microflora but there were numerically higher counts of *Lactobacillus* spp. and lower coliform and *Clostridium* spp. counts in the feces of pigs fed the direct-fed microbial compared to the other pigs fed the other treatments. Pigs offered supplemental *Lactobacillus* spp. in the water prior to and during an *Escherichia coli* challenge had significantly lower *Escherichia coli* and aerobe counts and significantly higher *Lactobacilli* and anaerobe counts in the digesta and mucosa of most of the gastrointestinal segments compared to pigs not receiving the direct-fed microbial (Huang et al., 2004). In a second experiment, similar microbial counts were found in pigs fed supplemental *Lactobacillus* spp. in the absence of carbadox (Huang et al., 2004).

Other microbes are also utilized as direct-fed microbials in the diets of pigs. *Bifidobacteria* have been reported to be effective in the reduction or prevention of diarrhea through the suppression of pathogenic bacterial colonization in the intestinal tract (Fuller, 1989). In addition, such bacteria as *Bacillus cereus* (Jadamus, 2001), *Brevibacterium lactofermentum* (Toride et al., 1998), *Lactobacillus casei* (Cho et al., 1992), *Bacillus cereus* (Zani et al., 1998), *Lactobacillus* spp. (Hale and Newton, 1979; Muralidhara et al., 1977; Hill et al., 1970; Tangtaweewipat et al., 2003), *Bacillus toyoi* and *Bacillus licheniformis* (Kyriakis et al., 1999) decreased incidence and severity of diarrhea in piglets. In contrast, dietary inclusion of *Bifidobacterium globosum A* (Apgar et al., 1993) and *Lactobacillus* spp. (Santos et al., 2002) did not decrease incidence of diarrhea in weanling pigs.

Supplementation of *Pediococcus acidilactici* to a liquid diet of weaned pigs resulted in increased fecal *Lactobacillus* spp. populations but no difference in the counts of fecal *Escherichia coli* compared to the negative control pigs (Bontempo et al., 2004). Administration of *Streptococcus faecalis* to piglets promoted colonization by beneficial microbes and decreased the occurrence of pathogenic microbes such as *Salmonella* in the intestine (Ozawa et al., 1983). Otsuka et al. (2002) found no difference in total viable counts of fecal anaerobes but higher fecal Eubacteria and lower fecal *Clostridium* spp. counts in growing pigs fed a probiotic. In an effort to control post-weaning diarrhea, two diets containing different concentrations of *Bacillus licheniformis*, a diet containing *Bacillus toyoi* and a negative control diet were offered, with *Escherichia coli* K88 detected in all pigs on days 3 and 5 post-wean but by day 22

post-wean, there was no *Escherichia coli* detected in the pigs fed *Bacillus toyoi* or the higher concentration of *Bacillus licheniformis* (Kyriakis et al., 1999).

Yeast

Yeast naturally occurs on plant material and can be found among the enteric microflora in animals (Mathew et al., 1998). Enzymes, vitamins and other nutrients contained within yeast have been proposed to produce beneficial performance responses in pigs (Kornegay et al., 1995). It has been proposed that yeast provides protection against invading pathogens by binding both the toxins and pathogens to their surfaces and by stimulating the immune system (Mul and Perry, 1994).

A pelleted or non-pelleted diet containing *Saccharomyces cerevisiae* had no effect on the ileal digesta counts of *Escherichia coli*, *Lactobacilli* and *Streptococci* in ileal-cannulated pigs during a 24 day experiment (Mathew et al., 1998). Upon euthanasia at 41 days of age, there was no effect of diet on the numbers of *Escherichia coli*, *Lactobacilli* and *Streptococci* attached to the ileal tissue (Mathew et al., 1998). In an experiment in which diets included *Saccharomyces cerevisiae* in the presence or absence of oat products, the yeast in combination with oats reduced the counts of fecal *Lactobacilli* while in the absence of oats, yeast had no effect on the counts of fecal *Lactobacilli* in weanling pigs (van Heugten et al., 2003). In a second experiment conducted by van Heugten et al., (2003), *Saccharomyces cerevisiae* in the presence or absence of a diet containing zinc, copper and antibiotics, reduced the counts of fecal total bacteria and *Lactobacilli* in weanling pigs. Overall, yeast supplementation had limited effects on microbial counts in fresh feces (van Heugten et al., 2003).

Prebiotics

A prebiotic is defined as 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 (Gibson and Roberfroid, 1995). It has been further defined as a carbohydrate that results in changes in numbers of key bacterial genera in the colon, i.e. *Bifidobacteria, Bacteroides, Lactobacilli* and *Clostridia* (histolyticum subgroup) (Olano-Martin et al., 2002).

There are four widely recognized criteria for the classification of prebiotics: a prebiotic 1) must not be hydrolyzed or absorbed in the upper gastrointestinal tract; 2) must be selectively fermented by one or more beneficial bacteria in the colon; 3) must alter the composition of the colonic microbial population towards a composition deemed to be healthier; and 4) must induce effects that are beneficial to host health (Gibson, 1999; Gibson and Fuller, 2000). Prebiotics include non-digestible oligosaccharides such as fructooligosaccharides (FOS), galacto-oligosaccharides (GOS), transgalactooligosaccharides (TOS), isomaltooligosaccharides, xylooligosaccharides, soybean oligosaccharides and inulin (plant storage carbohydrate) among others (Grizard and Barthomeuf, 1999).

Once ingested and present in the intestinal tract, these non-viable dietary components fortify certain components of the intestinal flora such as *Bifidobacteria* and *Lactobacilli* (Hidaka et al, 1986; Fukuyasu et al., 1987; Gibson, 1999). The growth of beneficial microbes, due to indigestible oligosaccharides, allows the microbes to control the intestinal pH through release of acetic and lactic acids (Moeller et al., 1990). These acids lower the intestinal pH to levels below those at which pathogens are able to effectively compete, and thus restricts the growth of many pathogenic bacteria (Rasic, 1983).

Similar to other dietary feed additives, there have been mixed results regarding the impact of prebiotics on gastrointestinal microflora. Several studies have reported no effect of dietary oligosaccharides on gastrointestinal and fecal microbial populations in piglets (Farnworth et al., 1992; Bolduan et al., 1993; Howard et al., 1995). Mikkelsen et al. (2003) reported no effect of dietary FOS and TOS compared to the negative control diet, on the numbers of fecal *Bifidobacteria, Lactobacilli*, coliforms, lactic acid bacteria and total culturable bacteria in weanling pigs. However, there was a higher number of yeast recovered in the feces of pigs fed FOS or TOS compared to pigs fed the negative control diet (Mikkelsen et al., 2003). Similar increases in the number of yeasts were reported in the ileum, cecum and colon of weanling pigs fed FOS and throughout the gastrointestinal tract of weanling pigs fed TOS compared to the negative control fed pigs (Mikkelsen and Jensen, 2004). There were no reported effects of either prebiotic on the numbers of *Bifidobacteria, Lactobacilli,* Enterobacteria and culturable anerobes (Mikkelsen and Jensen, 2004). Smiricky-Tjardes et al. (2003) reported that in ileal-cannulated grower pigs, TOS

increased the fecal concentration of *Bifidobacteria* and *Lactobacilli,* while soy solubles (source of GOS) further increased the fecal concentrations of both *Bifidobacteria* and *Lactobacilli* beyond those numbers seen in pigs fed TOS and the negative control pigs. Both TOS and soy solubles are reported to be bifidogenic (Moeller et al., 1990). In contrast, Gabert et al. (1995) found no difference in the ileal digesta concentration of *Lactobacilli* or other bacterial populations in pigs fed TOS or lactitol. This was likely due to the site of sampling, as both oligosaccharides and lactitol are readily fermented in the cecum and large intestine, where they promote the growth of *Bifidobacterium* spp. and *Lactobacillus* spp. (Gabert et al., 1995).

Other sources of prebiotics besides oligosaccharides have been evaluated for their effect on the gastrointestinal microflora. In a comparison of the effect of lactose and inulin on the weanling pig microflora, it was found that the diet containing a higher concentration of the lactose product increased the counts of *Lactobacilli* and decreased the number of coliforms in the cecum and colon whereas the diets containing either a lower concentration of lactose or a combination of inulin and the high concentration of lactose had no effect (Pierce et al., 2004). Konstantinov et al. (2004) evaluated the effect of a highly fermentable diet (high concentrations of lactulose, inulin, sugar beet pulp and wheat starch) compared to a low fermentable diet on the microbial population in weanling pigs. The bacterial diversity in the colon of pigs fed the highly fermentable diet was greater than in pigs fed the low fermentable diet, with higher numbers of *Lactobacilli* and *Enterococci* in the ileum (Konstantinov et al., 2004). In addition, there was an apparent stimulation of a *Lactobacillus amylovorous*-like population along the gastrointestinal tract of the pigs fed the highly fermentable diet (Konstantinov et al., 2004).

Wang et al. (2004) reported an enhanced microbial fermentation in ileal-cannulated pigs fed diets supplemented with either sugar beet pulp or wheat bran. Mushrooms that contain beta-glucans can also serve as dietary prebiotics. The inclusion of Lentinan, extract of *Lentinus edodes* mycelium (Shiitake mushroom), or dried *Lentinus edodes* mycelium powder was compared to avilamycin for their effects on the microbial population of weaned pigs (van Nevel et al., 2003). Only the diet containing the dried mycelium powder consistently had lower viable counts of total bacteria, *Escherichia coli, Streptococci* and lactic acid bacteria in the digesta of the stomach, distal jejunum and cecum as well as the jejunum mucosa (van Nevel et al., 2003). The inconsistent results from the mycelium extract may have been due to its lower concentration in the diet (van Nevel et al., 2003). Similar to the oligosaccharides, other prebiotics have also had mixed effects on the gastrointestinal microflora.

Mannanoligosaccharides

Mannanoligosaccharides (MOS) are derived from the outer cell wall of yeast. They are not considered to be direct-fed microbials due to the fact that they are not live organisms. They are also not considered to be prebiotics because research has failed to show either an *in vitro* or *in vivo* fermentative effect of this oligosaccharide. However, since they are oligosaccharides and have been reported to have an effect on the gastrointestinal microflora, they will be included in this section of dietary additives.

The *in vitro* theory regarding the action of MOS is that the mannan portion of the structure can specifically inhibit Type I fimbriae-mediated association of *Escherichia coli* (Ofek et al., 1977) and *Salmonella* (Linquist et al., 1987) with the mucosal surface of the intestine. It has been suggested that MOS may stimulate cytokine release, thus enhancing the immune system and improving the pig's ability to resist pathogenic bacterial challenges (Spring and Privulescu, 1998).

In an evaluation of the effect of MOS on the fecal microbial populations of weanling pigs, there was no significant difference between MOS, antibiotics and their combination on the counts of total aerobic bacteria and total coliforms (Lannon, 2002). In contrast, White et al. (2002) found that MOS tended to reduce fecal counts of total coliforms in weanling pigs at days 14 and 28 of the experiment. However, on day 14, there were no significant differences in the fecal numbers of *Escherichia coli, Lactobacillus* spp., *Bifidobacterium* spp., *Clostridium perfringens,* total aerotolerant anaerobes and total coliforms (White et al., 2002). In a second experiment conducted by White et al. (2002), weanling pigs were fed diets containing carbadox, MOS or neither additive and then challenged on day 30 with *Escherichia coli* K88. The pigs fed MOS consistently shed fewer total coliforms in their feces throughout the challenge period compared to pigs fed the other diets (White et al., 2002). Both MOS and carbadox significantly

reduced the numbers of total coliforms in both the jejunum and cecum compared to pigs fed the negative control diet (White et al., 2002). Encouraging results regarding the effect of MOS on the gastrointestinal microflora present in the digesta and mucosa contents of nursery pigs, is currently being evaluated through 16S rDNA/denaturing gradient gel electrophoresis in our laboratory (Miguel, unpublished data).

Plant Extracts: Essential Oils and Herbs

Plant extracts have been utilized for several years as a natural remedy to ameliorate or prevent the effects of a variety of ailments in humans. Some plant extracts, including essential oils, herbs and spices are known to contain compounds with antimicrobial effects (Arora and Kaur, 1999; Mellor, 2000; Varley, 2002; Wenk, 2003). The extracts can act as bacteriostats (prevent bacterial replication) or bacteriocides (kill bacteria) (Varley, 2002). Essential oils are extracted from plants by steam distillation or solvent extraction (Mellor, 2000). Herbs are non-woody, flowering plants; spices are aromatic substances of vegetable origin and botanicals are drugs made from a part of a plant such as roots, leaves or bark (Wenk, 2003).

Essential oils may contain such compounds as tannins, flavenoids or mucilages which can reduce the incidence of diarrhea due to their anti-inflammatory properties (Mellor, 2000). Several Chinese herbs have been reported to possess antimicrobial activities (Wenk, 2003). Phenols, found in the extracts carvacrol and thymol, alter membranes of *Salmonella* and *Escherichia coli* and other pathogens and cause the cell to die while many of the beneficial microbes are unaffected (Mellor, 2000). Of the essential oils available for dietary supplementation, oregano oil has had promising results. Oregano oil, which contains carvacrol, a bacteriostatic component, has been shown to significantly reduce post-weaning diarrhea in pigs by inhibiting intestinal *Escherichia coli* (Mellor, 2000). Oregano oil has a high antibacterial effect against both gram positive and gram negative bacteria (Smink, 2003) which makes it a valuable dietary supplement.

The mode of action of plant extracts is structural (cell wall or cell membrane) rather than genetic (DNA or protein synthesis), so microbes can not develop resistance to them as they can to antimicrobials. Plant extracts containing tannins or sharp substances are associated with increased production of pepsin and gastric acid, which thereby lowers the pH and creates a favorable environment for lactic acid bacteria and suppresses the growth of potential pathogens (Mellor, 2000).

Microbial Analysis of the Gastrointestinal Tract

The gastrointestinal tract of the pig is considered to be the home to a diverse array of microbes (Table 1). The inhabitants become more numerous and diverse as one moves from the stomach through the large intestine of the pig. Due to the low pH and acidic nature of the stomach, the quantity of microbes is relatively low with numbers estimated to be between 10^3-10^5/g contents (Gaskins, 2001). The proximal portion of the small intestine (duodenum and jejunum) has similar numbers to that of the stomach largely due to the rapid transit of digesta which prevents the colonization of microbes. The quantity of microbes is larger in the terminal portion of the small intestine and the hindgut. There is an increase from the ileum (10^8-10^9/g digesta) to the cecum (10^9-10^{10}/g digesta) and then the large intestine (10^{10}-10^{11}/ digesta) (Jensen, 1988) mainly due to the static nature of the digesta/luminal contents in this portion of the gastrointestinal tract. In these segments, the flow rate of the contents does not exceed the doubling rate of the microbial population levels (Savage, 1977), which allows for the colonization and proliferation of numerous microbes. The identity of these microbes has been and continues to be of great importance in relation to the formulation of diets, growth performance, prevention/diagnosis/treatment of disease.

In the past and less frequent now in the present, microbial analysis was cultivation-dependent. Samples were collected, diluted in the appropriate solution(s) and plated on selective or differential media for subsequent growth and identification of specific microbes. This procedure was and still is considered to be time-consuming, labor intensive and allows only for the growth of readily cultivable microbes which often gives a biased view of the microbial population. Many of the strictly anaerobic microbes are difficult to cultivate and thus are not detected through this technique (Tannock, 2001). Therefore, culture-based analysis may overestimate the numbers of certain groups (*Lactobacilli* and *Streptococci)* while failing to identify the abundant diversity present in the gastrointestinal tract of the pig (Pryde et al., 1999).

16S rDNA PCR-DGGE

In the mid to late 1990's, a new approach for the analysis of microbial diversity in the pig was first utilized. This approach moves from a culture-based to molecular-based microbial technique. This technique known as 16S rDNA PCR-denaturing gradient gel electrophoresis (DGGE) is based on isolation of DNA (Tsai and Olson, 1991, 1992) followed by 16S rDNA amplification by PCR (Muyzer et al., 1993) and then electrophoresis of PCR-amplified 16S rDNA fragments in a polyacrylamide gel containing a linear gradient of DNA denaturants (Muyzer et al., 1993). The gradient allows for the separation of DNA fragments of the same length but with different base-pair sequences (Muyzer et al., 1993). The fragments separate into bands or amplicons indicative of the number of bacterial species or assemblages of species (if more than one species migrated at the same rate) which allows for visualization of the genetic diversity of bacterial populations (Simpson et al., 1999). The number, precise locations in the gel, and intensities of the bands reflect the number and relative abundance of dominant rRNA sequence types in the sample which allows for the comparison of microbial communities within samples from one animal or between animals (Konstantinov et al., 2004). Thus, DGGE provides profiles based upon genetic diversity in microbial communities.

DGGE has both disadvantages and advantages to its application of microbial diversity analysis. A disadvantage to DGGE is that co-migration of DNA fragments, which may consist of 2 or more different bacterial species, may prevent the retrieval of clean sequences from individual bands (Muyzer and Smalla, 1998). Another limitation is that only relatively small fragments, up to 500 base pairs, can be separated, which limits the amount of sequence information (Myers et al., 1999). Also, DGGE does not provide actual bacterial numbers, but does provide an assessment of the relative similarity between samples. An advantage to the utilization of DGGE is that it has a high degree of sensitivity and allows for the detection of bacterial species that comprise 1% of the total microbial community (Muyzer and Smalla, 1998; Zoetendal et al., 1998). Identification of species can be performed following the excision of bands from the polyacrylamide gel, reamplifying the bands as described for PCR-DGGE followed by sequencing and then a search of sequences in a database using the BLAST search tool (Collier et al., 2003).

According to Simpson et al. (1999), DGGE can be utilized to monitor changes in bacterial populations throughout the pig's gastrointestinal tract, with differences being seen between animals in relation to diet, segments of the gastrointestinal tract and type of sample: mucosa or lumen. Collier et al. (2003) reported that tylosin and an antibiotic rotation (wk 1: 250 ppm CSP-250; wk 2: 30 ppm bacitracin + 4 ppm anticoccidial rozarsone; wk 3: 20 ppm lincomycin; wk 4: 25 ppm carbadox; wk 5: 10 ppm virginiamycin) initially reduced and homogenized the ileal lumen microflora of 15-35 kg ileal cannulated pigs with specific alterations observed in *Bacillus* spp., *Lactobacillus* spp. and *Streptococcus* spp. Simpson et al. (2000) found that individual pigs, some of which were dosed with *Lactobacillus reuteri* strain MM53, maintained a unique fecal bacterial population that was stable over time and perhaps influenced strongly by the host pig.

Due to the complexity of the intestinal microbial community, a majority of the microbial species inhabiting the gastrointestinal tract of the pig have yet to be isolated and characterized (Leser et al., 2002). However, 16SrDNA/DGGE has enabled some of the previously uncultivatable microbes to be identified (Leser et al., 2002). With the increasing popularity of this molecular technique, more information regarding the identification of these unknown microbial species should be made available in the future. As summarized by Simpson et al. (2000), 16S rDNA PCR-DGGE provides some of the tools essential for the study of host-microbe interactions. These interactions will become of greater importance as swine production moves away from the utilization of dietary antimicrobial growth promoters.

Other Microbial Analysis Procedures

There are quantitative measurements of microbes that can be performed with procedures other than 16S rDNA PCR-DGGE. These procedures include 16S rDNA PCR-TGGE, real-time quantitative PCR and fluorescent in situ hybridization.

The concept of 16S rDNA PCR-TGGE is similar to DGGE with the exception that the 16S rDNA fragments separate on a polyacrylamide gel according to a gradient of increasing temperature down the gel (Zoetendal et al., 1998).

Real-time quantitative PCR (qPCR) can measure the quantity of a specific bacterial species or the total bacteria within a sample. 16S rDNA is amplified

by PCR (Deplancke et al., 2002) in the presence of SYBR Green, which fluoresces when bound to double-stranded DNA. The intensity of this fluorescence is proportional to the quantity of amplified bacterial DNA of interest. Through the use of software that generates a standard curve vs. log DNA concentration for all standards, the DNA concentration of unknowns is determined by interpolation (Collier et al., 2003) and hence the intensity of the specific bacteria is quantified. An advantage of qPCR is that it offers high sensitivity and is more rapid than conventional PCR due to the avoidance of a post-PCR processing step (Wise and Siragusa, 2005). Using qPCR, Collier et al. (2003) reported tylosin and an antibiotic rotation (described previously) reduced the numbers of total bacteria, while the percentage of *Lactobacilli* increased by day 14 through day 28 in the ileal contents of pigs fed tylosin.

Fluorescent in situ hybridization (FISH) performed with 16S rRNA-targeted oligonucleotide probes has been shown to be a powerful tool for detecting and quantifying uncultured bacteria (Zoetendal et al., 2002). Primarily fecal samples have been utilized with this technique, in which the sample is combined with specific probes and if matched, the bacteria will fluoresce and can be quantitated when observed with a microscope. A disadvantage of FISH is that bacteria growing in natural environments tend to grow slowly and have small amounts of rRNA (target for fluorescent probes), which when labeled with the probe can often produce a dim or undetectable fluorescent signal. This can result in low percentages of fluorescent cells and thus an inaccurate underestimation of bacteria (Ouverney and Fuhrman, 1997; Watanabe et al., 2002). An advantage is that FISH allows for accurate and rapid identification of microbial populations, including those that comprise only about 1% of the total community, since it is based on molecular markers (Harmsen et al., 1999; Zoetendal et al., 2002). In addition, FISH allows for the identification of individual organisms and the quantitation of organisms in mixed communities without the need for isolation in pure cultures (Ouverney and Fuhrman, 1997).

Future of microbial analysis

With the advent of new techniques in the present and future, molecular microbial analysis will likely surpass or completely replace microbial culture techniques for the qualitative and quantitative evaluations of gastrointestinal microflora. Culture techniques will undoubtedly still be utilized for the isolation and growth of individual bacteria, but for enumeration purposes, molecular techniques far surpass those that involve cultivation. The utilization of DNA microarrays is gaining popularity and appears to be where the future of microbiology is heading. These techniques will allow us to gain a better and more thorough understanding of the delicate relationship between the host pig and its gastrointestinal microflora.

References

(The references cited can be requested from the authors)

Table 1. Predominant bacteria (%) isolated from the pig gastrointestinal tract.

Isolate	Total	Ileum	Cecum	Colon
Enterobacteria	24.5	53.4	10.4	5.8
Streptococci	22.8	32.2	18.8	16.8
Bacteroides	17.7	0.7	30.7	23.5
Eubacteria	6.3	0.7	8.5	10.3
Lactobacilli	5.9	3.4	5.6	8.9
Peptostreptococci	5.1	0.5	3.2	11.9
Fusobacteria	4.6	0.2	9.0	5.1
Selenomonads	3.3	1.2	5.9	3.0
Ruminococci	1.8	0.0	3.4	2.2
Clostridia	1.5	2.7	1.5	0.3
Scarcina	1.3	3.1	0.2	0.4
Megasphera	1.0	0.0	0.5	2.5
Butyrivibrios	0.8	0.0	1.3	1.1
Propionibacteria	0.4	0.0	0.9	0.4
Bifidobacteria	0.2	0.5	0.0	0.0
Veillonellae	0.1	0.0	0.2	0.0
Not characterized	**0.5**	**0.2**	**0.4**	**1.1**

Total (1679 isolates); Ileum (579 isolates); Cecum (529 isolates); Colon (571 isolates)

Adapted from Jensen (1999)

DIETARY FIBER FOR SOWS

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Summary

Dietary fiber components in feed ingredients are very diverse, thus it is difficult to establish specific nutrient requirements of fiber for swine. Digestibility of feed is inversely related to lignin content and information regarding the energy utilization of fibrous ingredients for sows is limited. Weighted averages of several experiments indicate that gestating sows fed high fiber diets have 0.3 more pigs weaned per litter compared to standard corn-soy diets. The mechanisms by which fiber improves litter size are unknown and specific amounts and types of fiber needed to elicit a response in litter size have not been determined. Feed costs per sow versus feed cost per ton of feed must be considered in the economic evaluation of high fiber diets, since larger quantities of fibrous feed per day are needed to meet nutritional needs. Swine producers may improve sow productivity, sow welfare, and profitability by using more fibrous ingredients in gestation feeds.

Introduction

As global population increases competition for nutrient dense grains to produce food and alternative fuels intensifies. Specialty products derived from food and alternative fuel processes of grains continue to expand and co-products of these processes often are used in animal feeds. Typically, such products are higher in crude fiber than the original grain. Gestating sows are well-suited to utilize less energy dense diets than growing pigs or lactating sows. Several experiments indicate improved reproductive performance of gestating sows fed high fiber diets compared to typical corn-soy diets. Expanded use and application of high fiber ingredients in swine feeds will reduce competition for grains and may improve profitability for swine producers if used appropriately. The intent of this report is to review the characteristics of dietary fiber and its impact on sow productivity.

Fiber Terminology: The definition of dietary fiber has been controversial for many years. Determination of crude fiber is not a precise analytical procedure. Crude fiber by definition is the sum of lignin and polysaccharides that are not digested by endogenous secretions of the digestive tract (Trowell et al., 1976). Dietary polysaccharides are primarily starch and sugars and are almost completely digested by monogastrics, whereas non-starch polysaccharides (NSP) are less digestible. Dietary fiber and NSP are often used interchangeably in terminology. The components of NSP are diverse, but generally are made up of cellulose, hemicellulose, and lignin. Quantification of NSP is based on solubility and neutral detergent fiber (NDF) is an estimate of total plant cell wall consisting primarily of cellulose, hemicellulose and lignin. Acid detergent fiber (ADF) is an estimate of cellulose and lignin. Perhaps the more appropriate measures of NSP for swine are soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) since they account for water-soluble NSP such as pectins, β-glucans, fructans and other soluble sugars. The sum of SDF and IDF equals total dietary fiber (TDF). Total dietary fiber is primarily used in the food industry and analysis is based on gravimetric procedures developed by Prosky et al. (1988). Limited information is available on the SDF and IDF composition of common feed ingredients for swine. Table 1 lists the fiber composition of some common feed ingredients.

Fiber Digestibility: Both NDF and ADF are listed in the Composition of Feed Ingredients Tables in the most current Nutrient Requirements for Swine (NRC, 1998). Although common feed ingredients for swine contain varying quantities of NDF and the ratio of cellulose, hemicellulose, and lignin vary in a given ingredient, estimates of dietary energy contributions from NDF can be calculated (Le Goff and

Noblet, 2001). In general the contribution of energy from NDF is doubled for sows over growing pigs (6.8 kJ DE per g of NDF for sows vs 3.4 kJ DE per g for growing pigs). Digestibility of fiber fractions (Table 2) differs between growing pigs and sows and the magnitude of difference is influenced by source of fiber (Noblet and Bach-Knudsen, 1997). Dietary fiber also decreases the apparent fecal digestibility of other dietary nutrients, such as crude protein and fat (Noblet and Perez, 1993; Le Goff and Noblet, 2001), but this effect is less in heavier pigs and sows (Noblet and van Milgen, 2004).

Digestibility is inversely related to lignin concentration. Pectins, fructans, β-glucans, and other components of SDF increase viscosity of digesta which may reduce passage rate due to reduced gut motility which leads to less mixing of dietary components with endogenous enzymes (Cherbut et al., 1990). Increased viscosity of digesta due to SDF components may interfere with complete digestion in the small intestine. However, swelling associated with increased viscosity creates greater surface area for microbial fermentation of substrate in the hindgut and this partially explains the relatively high total tract digestibility of SDF (Noblet and Le Goff, 2001).

Digestion of IDF is dependent upon microbial fermentation in the hindgut and this can partially explain differences in fiber digestibility of growing pigs and sows (Noblet and Shi, 1993). Hindgut fermentation generated 17% and 25% of total digestible energy from the diet of growing pigs and sows (Shi and Noblet, 1993).

Net energy formulations more accurately estimate the energy needs of swine than values based on DE or ME. Net energy values for growing pigs and sows differ primarily due to dietary fiber utilization and more efficient hindgut fermentation of the sow. Very limited data is available that has determined and validated net energy values of feed ingredients and mixed feeds for sows (Noblet and van Milgen, 2004). Digestible energy value of some common ingredients for growing pigs and sows are presented in Table 3 (Sauvant et al., 2002).

More nutrient dense diets are better suited for the nutritional needs of lactating sows. Higher fiber diets are not recommended for lactating sows, particularly during heat stress conditions (Renandeau et al., 2003). Thus, research on use of higher fiber ingredients has focused on the gestating sow. Pregnant sows (Table 4) fed diets with various types of fiber, oat bran as SDF source, wheat straw as IDF source, and sugar beet pulp as a SDF/IDF source of fiber had varying impact on digestibility (Retina Flores, 2003). Sows fed the SDF source had similar energy and nitrogen digestibility as control sows fed a corn-soy diet whereas sows fed SDF/IDF or IDF diets had reduced energy and nitrogen digestibility.

Other Effects of Fiber: The effect of dietary fiber on metabolic heat production is variable, but most studies indicate that dietary fiber intake increases heat production (Noblet and van Milgen, 2004). In some studies dietary fiber reduced heat production and these discrepancies may be explained by animal behavior (reduced physical activity) or overall metabolism (Schrama et al., 1998). De Leeuw et al. (2004) concluded that non-pregnant sows fed a diet with higher amounts of fermentable NSP from sugar beet pulp stabilized glucose and insulin levels and reduced physical activity in limit-fed sows several hours after feeding indicating a prolonged feeling of satiety. A recent review by Johnston and Holt (2005) details studies related to the influence of high fiber diets on stereotypic behavior of sows. The overall conclusions were that specific formulations of high fiber diets can decrease stereotypic behavior of sows if diets contain at least 30% NDF and fed at levels to ensure nutrient requirements for reproduction are satisfied.

Dietary fiber increases size, structure and function of the gut as well as maintenance energy requirements (Kass et al., 1980; Baldwin et al., 1980; Pond et al., 1989). Increased energy demand of the gut is likely due to increased epithelial cell turnover associated with feeding fibrous feeds (Jin et al., 1994). Proliferation of epithelial cells is supported by butyrate which is generated by fermentation of dietary fiber in the hindgut (Montagne et al., 2003). The absorption of SCFA generated during hindgut fermentation stimulates absorption of sodium which causes re-absorption of water from the colon (Mosenthin et. al., 2001). Enhanced epithelial cell turnover, fermentation and body water retention due to dietary fiber reduce the potential for non-pathogenic diarrhea. The increased epithelial cell turnover rate due to dietary fiber stimulates production of mucins, which serve as an important defense against pathogens and this likely explains some of the protective effects of dietary fiber against gut pathogens (Montagne et al., 2003). Proliferation of beneficial bacteria in the gut of pigs due to microbial fermentation and pH changes associated with some fiber sources tend to limit the growth of pathogenic species and ultimately enhance health status of pigs (Gaskins, 2003).

Effects on Reproduction: Improvements in reproductive performance of sows fed high fiber diets have been noted in most experiments (Grieshop et al, 2001; Reese, 1997). Grieshop et al. (2001) summarized 20 experiments that investigated effects of high fiber diets on reproductive performance of gestating sows. In experiments that reported a positive response (13 of 19 experiments), the increase of pigs born alive per litter from sows fed high fiber diets ranged from 0.1 to 2.3 and the weighted average for improvement in litter size of pigs born alive across the 19 studies was 0.4 pigs. Reese (1997) summarized 24 experiments involving the feeding of high fiber to gestating sows and noted that the weighted average of pigs weaned per litter was increased 0.3 pigs (Table 5). Several different types of fiber ranging from highly SDF sources to highly IDF sources were used in these studies that elicited a positive response to litter size. Feeding 0.3 kg wheat straw per day with 1.8 kg of a corn-soy diet during gestation improved subsequent lactation feed intake of a corn-soy lactation diet, and increased pigs weaned per litter, but reduced sow weight gain during pregnancy and pig birth weight (Ewan et al., 1996). Fiber intake per se influences sow reproductive performance, but the mechanisms of this response are unknown.

Johnston et al. (2003) proposed two potential explanations for the positive reproductive effects of fiber fed to gestating sows: 1) lower daily energy intake of sows fed fibrous diets, particularly during early gestation may contribute to improved embryonic survival and 2) 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.

High energy intake of sows in early gestation increased clearance rate of progesterone which was detrimental to development and survival of embryos (Jindal et al., 1996; 1997). Sows fed fibrous diets may have consumed less energy during the critical early stages of pregnancy which enhanced embryo survival and subsequently resulted in increased litter size at birth.

Several studies have linked increased sensitivity of peripheral tissues to insulin in humans suffering from non-insulin dependent diabetes when their diets contained elevated levels of dietary fiber (Hjollund et al., 1983; Karlstrom et. al., 1984; Landin et al., 1992). Nestler et al. (1988) reported prolonged insulin secretion in healthy men fed a meal containing guar gum as a source of SDF. Elevated insulin can enhance ovulation rate (Cox et al., 1987) and increase subsequent litter size (Ramirez et al., 1993). If elevated fiber intake modulates insulin metabolism one could theorize that sows fed high fiber diets during the follicular phase of the estrus cycle could have higher ovulation rates that lead to larger litter size. Recent evidence reported by De Leeuw et al. (2004) that feeding sugar beet pulp to non-pregnant sows stabilized glucose and insulin levels adds credence to this theory.

The reoccurring improvement in litter size due to feeding higher fiber to gestating sows seems real but specific attributes of fiber that favor improved reproduction are difficult to distinguish. Reese (1997) attempted to estimate recommended NDF intake of sows to improve litter size based on source of fiber as follows: 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. Such recommendations have not been validated in recent research, but have merit as an approach to discovering the apparent attributes related to fiber and improved reproduction.

Practical Aspects: Several factors need to be considered in the development of fibrous diets for gestating sows. Diets should be formulated and fed at a level to meet nutritional needs of the gestating sow. Digestion coefficients are generally greater for sows than those obtained for growing pigs. Equations to predict energy content of feeds for sows based on chemical analysis are available (Noblet and Shi, 1993; Noblet and Le Goff, 2001). Feed processing (i.e., particle size and pelleting) can improve digestibility of some sources of fiber.

Addition of bulky, fibrous ingredients to the diet will dramatically change handling characteristics of feed and this can be difficult to manage in feed handling, processing and delivery systems both at the feed mill and the sow facilities. Although pelleting can reduce bulk density of feeds and also increase energetic value of the feed, some of the satiety effect from feeding fibrous diets may be loss (Johnston and Holt, 2005). Increasing the fiber content (particularly IDF content) of feed results in larger volume of manure to handle and may be a deterrent to use in some manure storage and handling systems.

The ultimate reasons for use of fibrous diets for sows are driven by economics and welfare consid-

erations. Economic analyses of the value of fibrous feeds for gestating sows (Reese, 1998) and cull sows (Shurson et al., 2003) provide a basis for evaluation of the benefits and costs of fibrous feeds. Feed costs per sow versus feed cost per ton of feed must be considered in the economic analysis, since higher quantities of fibrous feed per day is needed to meet nutritional needs. Estimates of improvements needed in pigs weaned per litter to justify higher feed requirements of sows fed high fiber diets are presented in Table 6 (Reese, 1998). Swine producers may be able to improve sow productivity and profitability by using more fibrous ingredients in gestation feeds.

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Table 1. Fiber composition of corn, soybean meal and other fibrous feedstuffs [a]

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
Beet pulp	19.8	54.0	33.0	65.6	11.7	53.9

[a] Adapted from Johnston et. al. (2003).

Table 2. Digestibility (%) of fiber fractions and energy in high-fiber ingredients in growing pigs (G) and adult sows (S) [a]

	Wheat bran		Corn bran		Sugar beet pulp	
Digestibility, %	G	S	G	S	G	S
Nonstarch polysaccharides	46	54	38	82	89	92
Noncellulose polysaccharides	54	61	38	82	89	92
Cellulose	25	32	38	82	87	91
Dietary fiber [b]	38	46	32	74	82	86
Energy	55	62	53	77	70	76

[a] Adapted from Noblet and Bach-Knudsen (1997) as shown in Noblet and van Milgen (2004).

[b] Dietary fiber = nonstarch polysaccharides + lignin.

Table 3. Digestible energy value of some ingredients for growing pigs and adult sows [a]

Ingredient	DE, MJ/kg [b]		DE (kJ)/g of indigestible organic matter [c]
	Growing pig	Sows	
Wheat	13.85	14.10	3.0
Barley	12.85	13.18	2.5
Corn	14.18	14.77	7.0
Pea	13.89	14.39	6.0
Soybean meal	14.73	15.61	8.0
Rapeseed meal	11.55	12.43	3.5
Sunflower meal	8.95	10.25	3.5
Wheat bran	9.33	10.29	3.0
Corn gluten feed	10.80	12.59	7.0
Soybean hulls	8.37	11.46	8.0

[a] Adapted from Sauvant et al. (2002), as shown in Noblet and van Milgen (2004).

[b] As-fed basis.

[c] Difference between sow and growing pig (Noblet et al., 2002, 2003).

Table 4. Effects of diets high in SDF and IDF on energy and N digestibility [a]

Item	Control (corn-soy)	SDF (34% oat bran)	IDF (12% wheat straw)	SDF/IDF (16% beet pulp)
Feed intake, g/d	1826[y]	1870[xy]	1961[v]	1915[vx]
Sow wt. gain, g/d	315	302	313	334
Fecal DM, g/d	180.0[y]	175.3[y]	346.2[v]	207.9[x]
Urine, g/d	5707	7700	4754	7006
App. DE, %	87.9[x]	89.3[v]	82.7[z]	86.8[y]
App. N, %	86.1[v]	86.2[v]	82.8[x]	82.8[x]

[a] Renteria Flores, 2003.

[vxyz] Means within a row with uncommon superscripts differ (P<.01).

Table 5. Summary of changes in sow and litter performance due to added fiber observed in a wheat straw study and previous studies (adapted from Reese, 1997).

	Wheat straw [a]	Others [b]
Gestation intake, Mcal ME/d	0	-0.1
Gestation weight gain, kg	-4.1	-2.7
Lactation weight loss, kg	0	-1.4
Lactation feed, g/d	136	273
% completion [c]	0	10
Pigs born alive	0.5	0.3
Pigs weaned	0.7	0.3
Pig birth weight, g	-45	-90
Pig weaning weight, g	- 318	409

[a] Ewan et al., 1996.

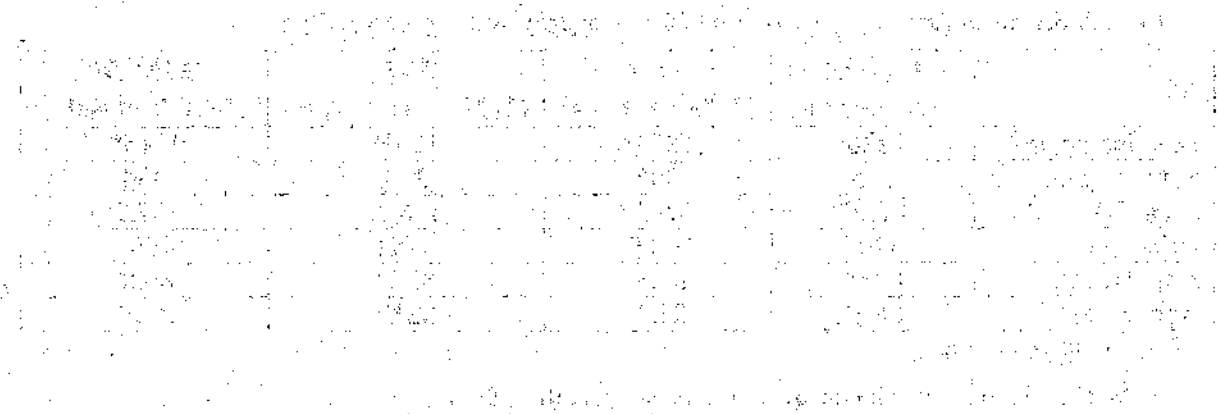
[b] Data from 20 references representing 14 fiber sources and over 1,113 litters produced from sows fed control and high-fiber diets during gestation.

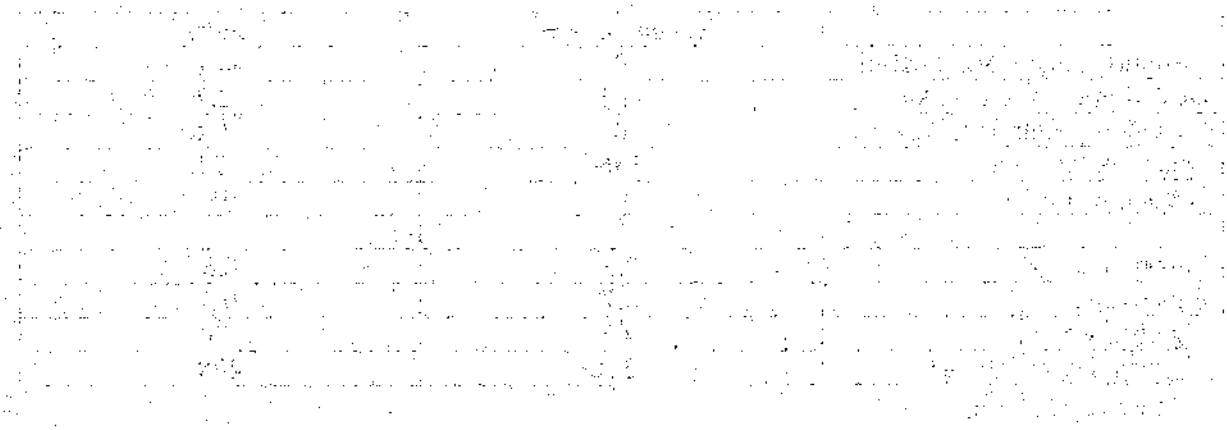
[c] percentage of sows that completed the experiments.

Table 6. Increase in pigs weaned per litter needed to offset extra sow feed expense [a]

Feed cost per sow increase, $/ sow/110 d gestation period	Value per weaned pig, $/pig		
	20	30	40
2	.11	.07	.06
4	.23	.15	.11
6	.34	.23	.17

[a] Adapted from Reese (1998).





Organic and Inorganic Copper, Zinc, Iron and Manganese

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Summary

It is very difficult to compare studies in which organic and inorganic mineral sources are studied. While there are several studies comparing zinc (Zn) sources at pharmacological concentrations, there are few in which the source is added to meet physiological needs. There is a need to evaluate sources of iron (Fe), copper (Cu) and manganese (Mn) in most stages of production. No studies comparing organic and inorganic minerals have investigated the important three way interaction of Cu, Zn and Fe. There is indirect data to suggest that amino acid associated trace elements may be absorbed via amino acid transport mechanisms and thus escape any specific trace element uptake regulation provided in the gut.

Introduction

Linkages between agriculture, nutrition and health are essential in preventing micronutrient deficiencies around the world that would alter human health (Welch and Graham, 2005). The World Health Organization (WHO) reports that when the food supply is inadequate and malnutrition occurs, there is increased susceptibility to disease and loss of life (WHO, 2002). So as we look to maintain healthy animals for meat, milk and wool production, we are also providing products to maintain and even improve the health of humans. The Food and Nutrition Board of the Institute of Medicine set Dietary Reference Intakes (DRIs) which include the Recommended Dietary Allowance (RDA) and Estimated Average Requirement (EAR), and Tolerable Upper Intake Level (UL) for all trace elements (Goldhaber, 2003). On the other extreme, the Environmental Protection Agency (EPA) and the WHO assess risk of toxicity. The EPA determines the highest dose level tested (in animals or humans) where an adverse effect does not occur (NOAEL) or the lowest dose where a critical adverse effect does not occur (LOAEL). Seldom do these agencies think about our production practices (i.e.: selection of mineral form) influencing human nutrient intakes and ultimately health. Also, in the past, we did not think about how choice of trace mineral source would influence composted swine manure or amended soils. However, today we know that composted swine manure (Liu et al., 2003) and material from anaerobic swine waste lagoons (Mueller et al., 1994) have increased Cu and Zn in soils. Thus, our selection of a trace mineral form is important not only for meeting the animal's needs, but for providing a nutritious product and not altering the environment.

Bioavailability

O'Dell (1997) defined bioavailability as "the proportion of the element consumed that is utilized for a biochemical or physiologic function". Thus, it is influenced by both absorption and assimilation. As noted by Underwood and Suttle (1999), bioavailability is influenced by the animal's age and species, the amount of the mineral in the diet, the form of the mineral, other dietary components and environmental factors. Thus, determination of bioavailability and comparison of bioavailabilities is not just looking at excretion or organ storage. Unfortunately, we often forget these basic biochemical principles.

Due to a limited number of quality studies in the published literature, let alone studies where animals are of the same species and age, we can draw few conclusions about bioavailability when comparing organic and inorganic minerals. Needless to say, this is confounded by the number of "organic" mineral forms that are available from suppliers. The Association of American Feed Control Officials (AAFCO) provides numerous ways to classify "organic" trace elements. However, evidence of the benefit of organic forms due to early availability in ruminant animals and resistance to the effect of phytate by non-ruminants may be a result of by-passing homeostatic regulation for some elements (Underwood and Suttle, 1999) and not differences in bioavailability.

To assess bioavailability, usually more than one nutritional status indicator related to the specific nutrient should be evaluated. In a bioavailability study, O'Dell (1997) noted that the animals must be fed a diet lacking the specific trace element before or during the study and be fed graded concentrations of this trace element. For many elements, good status indicators or biomarkers are not available. Commonly used indicators include growth rate, tissue element concentration, metal-dependent enzyme activity, metallo-protein concentration, chemical balance and pathologic signs (O'Dell, 1997). Hence, true physiological and biochemical utilization would not be evaluated in a growth study where tissue concentrations are measured.

Organic and Inorganic Sources

Nomenclature: In the 2005 Reference Issue and Buyers Guide of Feedstuffs magazine (2004), Cu sources include: amino acid complex, lysine, proteinate and sulfate. Iron sources include amino acid complex, carbonate, methionine, proteinate and sulfate. Manganese sources include amino acid complex, methionine, oxide, proteinate and sulfate. Zinc sources include amino acid complex, methionine, proteinate, oxide and sulfate. However, you may not even find your "favorite" source by one of these names. The ratio of ligands to specific minerals and the different thermodynamic stability of various ligands influence the liability of ligand mineral complexes and bioavailability, again indicating why extrapolation to different minerals or experimental conditions is risky (Vasconcelos et al., 1996).

Copper

Design factors to consider: When Cu studies are compared, it is essential to consider factors that may affect the results. Besides the influence of species, age, nutritional status, and physiological stage, other factors also alter many parameters.

When the concentration of dietary Cu is below requirement or in excess, it will alter the hemoglobin concentration in Fe studies (Ramirez-Cardenas et al., 2005). It has been observed in lambs that the number of daily feedings (1 vs. 4) alters the amount of Cu stored in the liver (Luo et al., 1996). It has been established that long term feeding of high or pharmacological concentrations of Zn depresses Cu status (Hill et al., 1983), but since we usually feed pharmacological Zn after weaning, it is essential that we consider Zn concentrations when evaluating Cu sources. In a rat study (Du et al., 1996) when Zn was adequate, hepatic Cu concentrations were different with two dietary sources of Cu. However, when 1,000 ppm of Zn was added to the diet, hepatic Cu was not altered by the dietary Cu sources. Du et al. (1996) also reported that amount of dietary Cu and Fe influence liver and spleen Cu concentrations, but only concentrations of dietary Fe altered renal Cu concentrations. The influence of other dietary antagonists of Cu bioavailability was demonstrated by Spears' laboratory (Spears et al., 2004a). They reported, in cattle, that two Cu sources respond differently with dietary adequacy of molybdenum (Mo) and sulfur (S) and the Cu status of the steers. When the steers were Cu depleted and the dietary Mo concentration was low, the Cu sources had different bioavailabilities. However, if Mo and S (Cu antagonists) were high in the diet, one of the sources provided more Cu for storage than the other.

It was recently reported by Aarestrup and Hasman (2004) that in an *in vitro* study, utilizing bacteria isolated from animals, *enterococci* showed an acquired resistance to Cu. They noted that *Salmonella* was less susceptible than other bacteria to Cu sulfate, Zn chloride and some disinfectants. This may indicate the *202 E. coli* may be evolving and may result in less control when pharmacological Cu is fed. It would be interesting to assess if this same observation would be true with organic forms of Cu.

Recent Cu form comparisons: Cromwell (1998) showed that at pharmacological concentrations (250

ppm) of tribasic Cu chloride resulted in performance similar to Cu sulfate in pigs. More recently, Spears et al. (2004a) reported estimated relative bioavailabilities with various Cu parameters. In steers, using multiple linear regression, the relative bioavailability of tribasic chloride was 1.96 with hepatic Cu, 1.18 with plasma ceruloplasmin and 1.32 with plasma Cu when compared with Cu sulfate (1.0). They also reported that tribasic Cu chloride was almost insoluble in water, but solubility was improved in 0.1% HCl. When this form of Cu was incubated with glycine and lysine at a pH of 3.0 or higher, the amount of soluble Cu increased. This increased solubility in acid pH may be due to Cu binding with amino acids, thus forming an organic Cu form.

In rats, Du et al. (1996) found that feeding Cu lysine resulted in greater liver and spleen Cu concentrations than Cu sulfate indicating improved bioavailability. On the other hand, Cu proteinate did not improve bioavailability. Both organic forms of Cu resulted in less renal Cu than Cu sulfate. Of interest, Cu lysine fed animals had greater hepatic Fe concentrations than those fed Cu sulfate, but Cu proteinate did not improve the concentration of this interrelated trace element.

Copper supplementation (10 or 20 ppm) was shown to improve cattle's ability to respond immunologically (Dorton et al., 2003). However, Engle's laboratory noted that the response was "variable and depended on the class of immune cells being studied as well as the source and concentration of the supplemental Cu". Copper amino acid complex resulted in higher antibody titers for steers than titers reported for steers supplemented with the same amount of Cu provided by Cu sulfate.

Apgar and Kornegay (1996) found that Cu lysine and Cu sulfate fed at 200 ppm to grow-finish pigs resulted in increased gain with the lysine source, but the absorption and retention were similar. Jondreville et al. (2003) note that "studies dealing with the assessment of organic sources of Cu or Zn within the context of lowered dietary supply are scarce". Perhaps due to economic concerns, organic and inorganic minerals are often fed together. Creech et al. (2004) compared the growth, nutrient status and excretion of gilts fed Fe, Cu, Zn and Mn (1) as sulfate in diets with concentrations above NRC (1998) and typical of concentrations fed in the industry, (2) reduced concentrations as sulfates and meeting the NRC's recommendations, or (3) reduced as half sulfates and half proteinates and at the same concentrations as treatment 2. This collaborative team was the first to report that feeding excessive (typical industry intakes) Fe, Cu, Zn and Mn reduced G/F in the nursery and ADG and FI during the developing period. Feed efficiency was improved in gilts fed the combination of inorganic and organic sources in the nursery compared with those fed the same amount of these trace elements as sulfates. All biomarkers of nutrient adequacy were within the acceptable range. Fecal Cu was reduced as dietary concentrations were reduced regardless of source. However, pigs fed the combination organic/inorganic diet excreted less Cu in the growing and developing phases than those fed the reduced inorganic diet.

With increased emphasis on the amount of Cu and Zn in manure, agronomists have studied the amount of available Cu and Zn to crops and their movement in soils. Recently, Zheljazkov and Warman (2004) reported that the amount of Cu and Zn in compost-amended soils may predict the amount of Cu taken up by some crops but not others. The impact of dietary form on the availability in compost and manure when placed on soils has not been evaluated.

Zinc

As with Cu, there are few well designed studies to compare Zn sources. Since Zn provided as oxide at pharmacological concentrations and adequate Cu has been shown to increase performance (Smith et al., 1997); (Hill et al., 2000); (Carlson et al., 1999), several studies have compared oxide with organic Zn at 2,000 to 3,000 ppm. When Carlson et al. (2004) fed up to 500 ppm of Zn as a polysaccharide, up to 800 ppm Zn as a proteinate and 2,000 ppm Zn as oxide for various lengths of time with all diets containing 165 ppm Zn from sulfate, they did not observe any performance differences. As expected, diets containing Zn from organic sources and fed at lower concentrations resulted in less fecal Zn. Research at the University of Kentucky where pigs were weaned and fed in pairs (Meyer et al., 2002) for 21 d showed that fecal Zn was increased by pharmacological dietary Zn concentrations.

The NCCC-42 swine nutrition committee compared feeding 2,500 ppm Zn from oxide with 125, 250, or 500 ppm Zn provided by Zn methionine and a control diet containing 125 ppm Zn (Hollis et al.). Pigs fed the pharmacological concentration of Zn oxide out performed the control pigs in ADG and ADFI.

When Zn was provided as methionine pigs grew faster and ate more feed than pigs fed the control diet (125 ppm Zn), but pigs consuming pharmacological Zn as oxide had higher ADG and ADFI than pigs fed Zn methionine. This committee also compared the performance of pigs fed 2,000 ppm Zn from oxide with that of pigs fed 500 ppm Zn from either oxide, polysaccharide complex, proteinate, amino acid complex, amino acid chelate or methionine and a control diet containing 139 ppm Zn. Pigs fed pharmacological Zn had higher ADG than pigs fed any of the other treatments.

Feeding 2,000 ppm Zn from Zn oxide or Zn methionine to nursery pigs for 14 d, (Rincker et al.) resulted in no difference in whole body Zn concentration (346.3 ppm). However, when pigs were fed 2,000 ppm Zn from Zn oxide for 21 d followed by 14 d of an adequate Zn diet, the concentration of Zn in the whole body was 72.3 ppm (Rincker et al., 2004). These results indicate that the body has the ability to excrete large amounts of fecal Zn to re-establish a "normal" body burden when pharmacological Zn is fed, regardless of dietary source. Fecal Zn of pigs fed Zn methionine or Zn oxide at 2,000 ppm began to increase at approximately 10 to 11 d after the feeding of pharmacological Zn was initiated. This is presumably when the body becomes "loaded" with Zn. Hepatic and renal Zn, Cu and Fe concentrations were not different in pigs fed pharmacological Zn as either oxide or methionine (Rincker et al.). Pigs fed Zn as methionine had higher urinary Zn which is an indirect measure of increased absorption perhaps by uptake by the amino acid transporter.

Schell and Kornegay (1996) compared pigs fed various pharmacological concentrations of Zn as methionine, lysine, oxide or sulfate with pigs fed 105 ppm Zn. They observed no differences in performance parameters. Utilizing serum Zn concentrations, bioavailability estimates were increased for methionine and oxide when 2,000 ppm were fed compared with 3,000 ppm while the bioavailability of Zn lysine was not changed. When liver Zn was utilized to estimate bioavailability, methionine's estimate increased at 2,000 ppm compared with 3,000 while oxide and lysine estimates of bioavailability decreased. Zinc sulfate was considered the standard at 100%. These data remind us that concentration and the test element, not just form, are important considerations in estimating bioavailability. While a large number of studies have attempted to assess bioavailability of Zn with pharmacological concentration using performance and tissue organ concentrations, this may not be appropriate. Limited data indicate that mode of action of pharmacological Zn provided as oxide to improve performance is probably not due to absorption of Zn but improvement of gut morphology (Carlson et al., 1999).

Because lysine is the first limiting amino acid in corn-soybean meal diets, and it is associated with a useful organic Zn product (Zn lysine), Cheng et al. (1998) investigated the addition of lysine to diets with added Zn as Zn lysine or sulfate (100 ppm Zn). Liver Zn was not altered by dietary lysine, but kidney and rib Zn were increased. This is again indirect evidence that lysine, an amino acid, may have improved Zn uptake from the gut perhaps via the amino acid uptake mechanism. Because diets were adequate in Zn, there may have been an increase in renal Zn resulting in increased urinary Zn (not measured). Growth performance parameters and Zn concentration in storage organs were not altered by Zn form.

Creech et al. (2004) reported that fecal Zn was reduced in all phases of the study in gilts fed the reduced inorganic and reduced combination treatment of sulfate and chelated Fe, Cu, Zn and Mn. Pigs fed the reduced combination treatment (sulfate and chelate) excreted significantly less fecal Zn during the nursery phase than gilts fed the reduced inorganic diet (sulfate source).

Wedekind et al. (1994) utilized three dietary concentrations of Zn sulfate to establish a standard curve where bone and plasma Zn concentrations were regressed on supplemental Zn intake. Using multiple regression, slope-ratio analysis, they then estimated the bioavailability from the bone and plasma Zn concentrations of pigs fed Zn as oxide, lysine or methionine. They reported the following estimate of bioavailabilities: Zn sulfate (used to establish curve) > Zn methionine > Zn oxide > Zn lysine. When dietary Zn was provided as sulfate or methionine, bone and plasma Zn were similar (Revy et al., 2004). Both were increased by the addition of phytase regardless of Zn source in pigs that were similar in age to those used by Wedekind et al. (1994). Thus, these studies again indicate that techniques and parameters used in evaluation of sources often give very different results.

Swinkels et al. (1996) compared Zn amino acid chelate and Zn sulfate relative to their ability to replete Zn status in pigs fed a low Zn diet for 24 d. No effect of Zn source was observed.

Predieri et al. (2005) synthesized stable metal chelates with divalent metals and methionine hydroxyl-analogues. Carrying out an *in vivo* trial with Zn-depleted rats, they compared Zn sulfate with their synthesized Zn methionine and found higher retention (61%) with the methionine source than with sulfate. Utilizing corn with high and normal methionine content, House et al. (1997) reported that rats absorbed equal amounts of Zn if their methionine intake was adequate. However, they found Zn absorption to be reduced with soy-based diets that were fed without additional methionine. Perhaps some of the differences in bioavailability that have been reported may not only be due to concentration of Zn in the diet but to amino acid concentrations that may become ligands during digestion.

Edwards and Baker (1999) and Cao et al. (2002) utilized chicks to estimate relative bioavailability of Zn. Edwards and Baker (1999) used sulfate as their standard and reported that feed grade oxide bioavailability ranged from 78 to 69% while analytical grade was 80%. Zinc metal dust and metal fume were reported to have an estimated relative bioavailability of 67 and 36%, respectively. Cao et al. (2002) used Zn acetate as their standard to compare with Zn proteinate and Zn methionine. In their study with "insufficient number of pens available to make a complete factorial arrangement of treatments with adequate replication", they reported that mucosal metallothionein was a useful parameter to estimate bioavailability of Zn because changes occur before excess Zn is deposited in bone and can be detected. Their relative estimates of bioavailability for Zn methionine were 0.88, 0.91 and 0.78 and for Zn proteinate were 1.1, 1.24 and 1.16 at 3, 6, and 9 d, respectively.

In rats, Yonekura and Suzuki (2003) found that feeding chitosan, alginic acid or raw potato starch as dietary fiber polysaccarides reduced the negative impact of phytic acid on Zn bioavailability. Perhaps this is part of the explanation for the use of Zn polysaccharide as an available organic Zn source.

Zinc glycine has been found to be a very available source of Zn in steers (Spears et al., 2004b), but there does not appear to be similar research in pigs. They reported that urinary Zn was higher when 20 mg per day was provided as Zn methionine or Zn glycine than when a similar amount was provided by sulfate. Hepatic Zn was higher in steers fed the glycine form of Zn than stores resulting from feeding other Zn sources; thus indicating that Zn glycine may be an available form of Zn. Of interest, perhaps from a mechanistic standpoint, Williams et al. (2004) reported that Zn histidine had a greater potency and bioavailability in cultured cortical neuron studies. Neurons pre-treated with Zn histidine had less damage from hydrogen peroxide-induced caspase-3 activation and c-jun-N-terminal kinase phosphorylation. Thus, cultured neurons were protected from oxidative insults and early apoptosis.

Iron

Pigs are known to have a high need for Fe shortly after birth due to the lack of Fe in milk and the rapid demand for Fe for growth and hence tissue expansion. Because of gut closure with 24 to 36 h of birth, Fe injections have become a common practice in the swine industry. Recent work by Rincker et al. (2004) clearly demonstrated that Fe provided by nursery dietary ingredients is not adequate to sustain Fe status and that 100 ppm Fe should be added to the diet from a highly available Fe source such as sulfate. However, the amount of Fe that should be added to grow/finish diets has not been studied with today's lean genetics.

In an attempt to determine if forms of dietary Fe could be solubilized and given orally to newborn pigs to prevent Fe deficiency anemia, Kegley et al. (2002) compared injectable Fe from gleptoferron given 12 h after birth with Fe from Fe methionine or sulfate given at birth or at 3 d of age. Injectable Fe increased liver and spleen Fe concentration 2 to 3 fold compared with oral doses. However, when only oral Fe sources were compared, 200 mg of Fe from methionine given on d 3 resulted in the greatest increase in plasma Fe, hemoglobin concentration and percent hematocrit at d 14 and 21. Use of Fe methionine as an oral preparation for prevention of Fe deficiency anemia deserves further investigation.

Schweigert et al. (2000) studied the impact of two different Fe dextran preparations on circulating vitamins A, E and C. They reported that anemia was prevented by both sources [iron (III)-hydroxide-dextran-heptone acid and iron (III)-hydroxide-dextran], and that vitamin C concentration in plasma was decreased by both at 10, 17 and 24 d after injection. Vitamin E concentrations were increased on d 10 compared with controls as were plasma retinal concentrations 24 d after injection compared with pigs not given Fe injections.

While Rincker et al. (2004) recently reported that 100 ppm Fe from ferrous sulfate would sustain

Fe status during the nursery phase, Yu et al. (2000) compared Fe sulfate with an Fe amino acid complex. They found that functional Fe parameters increased as the amount of dietary Fe amino acid complex was included in the diet, and provided a greater change for these parameters than an equal amount of Fe provided as ferrous sulfate. However, Fe stores in the liver, spleen and muscle were not altered by source. Interestingly, they found that the red color score "a" for skin color was higher when pigs were fed the Fe amino acid complex than a comparable amount of ferrous sulfate.

Creech et al. (2004) noted that fecal Fe was decreased when Fe was reduced in the diet regardless of source (sulfate vs. combination of sulfate and chelate).

In humans, 5.4% and 4.0% of ferric glycinate was found to be absorbed when 6 and 15 mg of Fe was given with milk. A similar amount was absorbed from ferrous ascorbate. Regardless of form, there was a negative correlation with serum ferritin indicating that Fe absorption was determined by the body's Fe stores (Pizarro et al., 1998). Ait-Oukhatar et al. (2002) profused duodenal rat loops in intact, live animals and reported that the absorption of Fe bound to the first 25 amino acids of caseiophosphopeptides was greater when compared with Fe ascorbate. This same group reported that absorption was similar between this milk protein product and ferrous sulfate when labeled sources were given to Fe-deficient females. Roe and Fairweather-Tait (1999) reported that the absorption of reduced Fe powder used to fortify flour in the UK was similar to ferrous sulfate. When three oral Fe compounds were compared, ferrous sulfate had greater bioavailability than ferrous glycine chelate or ferric EDTA (Ferreira da Silva et al., 2004).

When heme or hemin was added to casein-dextrose diets, hemin was found to be poorly available to the rat and unavailable to chicks, cats and dogs. Hemoglobin Fe was 68% available to rats, 93% to chicks, 90% to dogs and 70% to cats (Fly and Czarnecki-Maulden, 2000). These forms of Fe are those found in red blood cell and blood meal products used in swine diets. While soybean hulls are often used as fiber sources in swine diets, they have been shown to provide Fe to rat mothers that were Fe-deficient during gestation or lactation (Huh et al., 1999). It is well known that bioavailability of all Fe sources is increased when body Fe stores are decreased. The value of this as an Fe source in swine diets is unknown.

Manganese

Very little literature is available about Mn sources in livestock, experimental animal and human diets. Most recently Li et al., (2004) compared five Mn methionine sources with differing chelation strength with Mn sulfate. They reported that only cardiac Mn superoxide dismutase mRNA differed among the Mn source treatments. Relative to Mn sulfate they reported bioavailabilities of 99, 132, and 113% for Mn methionine sources that were weak, moderate and strong in chelation strength. When these same Mn sources were fed to broilers in diets with high Ca concentrations, they found the relative bioavailabilities compared with Mn sulfate (1) to be 1.12, 1.45 and 1.48 when using Mn superoxide dismutase mRNA which was more sensitive than cardiac Mn concentration. These were commercially available Mn sources, but were obtained from independent distributors and not the manufacturers.

Utilizing cell culture, Reaney and Smith (2005) suggested that the oxidation state of Mn is important in managing cytotoxicity. Cells exposed to Mn provided as chloride (II) contained less Mn than those exposed to Mn pyrophosphate (III) when concentrations were between 50 and 200 μM. However, at 10 and 50 μM, there was a higher percent of non-viable cells with Mn (II) than (III). This was reversed at 150 and 200 μM concentrations.

Creech et al. (2004) reported that less Mn was excreted in the reduced diets at all phases of production. During the nursery phase, pigs fed the combination dietary treatment of chelates and sulfates excreted less fecal Mn than those fed only Mn sulfate.

Research needs

Hostetler et al. (2003) hypothesized that organic minerals might be important in improving reproduction. However, these suggestions were based on opportunities not findings. With the known negative impact of free Fe on body health and the high concentration of Fe in diets, mostly as oxide, it is important to determine if Fe from an organic or inorganic source should be added to grow/finish diets. This is especially important in light of the findings of the influence of amino acid complex Fe on skin color (Yu et al., 2000) and the preference for specific pork color by certain foreign markets. With the increasing emphasis on reduced excretion for environmental management while protecting health and welfare and improving product contribution to human health, more well-designed studies are needed.

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Organic Selenium Fed to Swine—Its Potential Impact on Human Health Issues

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Summary

Selenium (Se) has had a widely differing reputation throughout world history. Its first role was identified as a toxic element, but during the mid 1950's it was then identified as a metabolic essential. Although Se per se does not provide benefits directly to the animal or human, it is when the element is incorporated into an amino acid (selenocysteine) where it is synthesized into various selenoproteins that provide antioxidant protection. During the past two decades research has recognized that Se may have an anti carcinogenic role in humans and may aid in the prevention of other human diseases and conditions. Although Na selenite and organic (i.e., selenized yeast) are both approved for addition to livestock diets, the feeding of organic Se to livestock increases the Se content of the muscle, milk and eggs produced that has potential benefit to the human. The consumption of animal products containing higher Se concentrations may be achieved when organic Se is fed and may be an avenue where the livestock industry can not only provide a good protein source to humans, but there may be health benefitsprovided from the Se retained in the animal products.

Introduction

Selenium (Se) has had a rather tumultuous history both world wide and in the United States. Marco Polo indicated in his writings, while in eastern Asia during the 13th century, that certain plants consumed by their horses and mules caused hoof and leg problems making traveling difficult. In the United States certain areas in the north central states produced "poison plants" that caused a "wasting condition" in livestock. The hooves of horses used by General George Custer during the late 19th century were thought to be affected by plants consumed when they were confined in pasture lots containing forages of higher Se contents. The famous "Little Big Horn" massacre was thought to influenced by the inability of the Army horse's to run faster than the Indian mounts, thus forcing General Custer and his troops to stand and fight overwhelming Indian odds (Anonymous, 1999). This battle is largely responsible for ultimately changing the future destiny of the Indian tribes, and the history of the United States.

Selenium was largely considered a toxic element prior to 1950 and a great deal of research conducted demonstrated the effects of Se toxicity (selenosis) on animals and humans. Research conducted by Nelson (1943) initially suggested that excess Se was carcinogenic, thus involving the federal government in regulating its dietary inclusion. In 1957 it was discovered that Se was in fact an essential mineral (Schwarz and Foltz, `957). Later work showed that its effect was as a component of various seleno enzymes necessary for the metabolism of nutrients in the cell. Subsequent research during the late 1960's and early 1970's clearly demonstrated that livestock on many farms suffered from Se deficiencies, thus resulting in FDA approving its supplemental inclusion (Na selenite or selenate) at dietary levels not to exceed 0.10 ppm (mg/kg diet), a level that was later amended to 0.30 ppm (FDA, 1974, 1987). More recently, the FDA (2002) has approved the incorporation of selenized yeast as an organic Se source for several livestock species. Research conducted during the past decade has now demonstrated that

Se supplements may in fact prevent the occurrence of cancer and other diseases in humans. This brief review outlines the essentiality of Se in nutrition, the effects of low or supplemental Se to the human diet, the effects of inorganic and organic Se when fed to swine, and implications for enhancing human health by providing Se through animal products, particularly pork. Much of the human research reported herein was from reviews or research conducted by Rayman (2000), Ip et al; (2002) or a briefing paper published by British Nutrition Foundation (2001).

The case for Se as an essential nutrient

Selenium as the element *per se* has no antioxidant protection activity, but when incorporated into the appropriate enzyme systems it is biologically active. Physiological functions ascribed to Se, expressed by a series of selenoproteins, can generally be categorized as having antioxidant protection capability. In general, these Se containing molecules are seleno-enzymes that contain selenocysteine as the essential component. A low Se intake and thus a low selenocysteine synthesis reduces the production and subsequent antioxidant activity in the body. Selenocysteine has been termed the 21st amino amid (Rayman, 2000), but cannot be considered a dietary essential amino acid because it can be synthesized by the body when provided with adequate Se. To date there have been approximately 35 selenoproteins identified, but about half have been associated with mammalian tissue generally having antioxidant activity (Table 1). This antioxidant activity generally prevents the accumulation of free radicals by removing accumulated hydrogen peroxide and lipid and phospholipid hydroperoxides in tissue, as well as having antioxidant activity in sperm cells, thyroid hormones, and aids in protecting the muscle cell from oxidative damage (Brit. Nutr. Foundation, 2001).

Unless unchecked, the accumulation of the various free radicals may damage the cell such that various systems or tissues of the body would become dysfunctional. Although Se has a vital role in maintaining antioxidant protection, the body has several other effective antioxidants, each having a specific function at specific locations both intracellular and extracellular. Under normal physiological conditions the antioxidant system has synergistic relationships. For example, within the cell there are two forms of superoxide dismutase (contains Zn or Cu and Mn), each being active in different metabolic processes, four forms of glutathione peroxidase (containing Se), catalase (contains Fe), vitamin E, Vitamin C, β-carotene, and other antioxidants. In addition, plant tissues contribute antioxidants (flavenoids, etc.) to the animals when consumed, that provide antioxidant protection. When one antioxidant is lacking or is produced in inadequate quantities, it is possible that the body cannot compensate and various diseases or tissue damage might ensue.

The Role of Se in Human and Animal Health

There is abundant information demonstrating that supplementing a diet that is at or above that necessary for selenoprotein synthesis further enhances the health and nutritional status of both animals and humans. Although part or all of this is ascribed to its antioxidant function, Se has been shown to improve the immunocompetence of animals and appears essential in maintaining an optimum healthy condition.

Low or borderline deficient Se status in humans may increase the virulence of some viruses and affect the metabolism of Se (Rayman, 2000). Such a condition is thought to lower plasma Se and subsequent lowering the production and activity of the Se containing enzyme glutathione peroxidase (GSH-Px1). In areas where grain and forage Se is low this has been shown to affect the incidence of cardiomyopathy. This low Se content in the foods consumed by the human may affect the genome of the virus infecting the human host that may ultimately affect the pathology of the heart and other body tissue. It has been speculated that new strains of influenza that occur frequently in the United States may originate in areas of China that are low in Se. The flu may thus may be the result of genetic mutations of viruses that may become more virulent in a Se deficient host and subsequently transferred in a more virulent state. Other viral diseases that have been shown responsive to Se supplementation are those associated with HIV/AIDS, and Hepatitis B and C. In case studies, a number have shown a decline in blood Se concentrations where higher cases of cancer are also reported (Brit. Nutr. Found., 2001). These viruses may use the available Se more effectively than the host, thus incorporating absorbed Se into viral selenoproteins, and preventing the host to mount an effective immune response (Rayman, 2000). Maintaining a high Se status may be the most effective way to "turn away" diseases in the human.

Table 1. Known functional selenoprotein in mammalian tissue. [a]

Mammalian selenoprotein	Function
Glutathione peroxidase (GSH-Px1, GSH-Px2, GSH-Px3, GSH-Px4)	Antioxidant (intracellular, lipoprotein, circulatory system, DNA)
Sperm capsule selenoprotein (GSH-Px 4)	Protects developing sperm cells from oxidative damage, structural role in nature sperm
Indothymine 5′-deriodinase (Type I, II, III)	Thyroid hormone metabolism
Thioredoxin reductase (TR1, TR2, TR3)	DNA synthesis and antioxidant activity
Selenoprotein P	Protects endothelial cells from peroxidative damage
Selenoprotein W	Muscle function
Selenoprotein synthetase	Selenoprotein synthesis

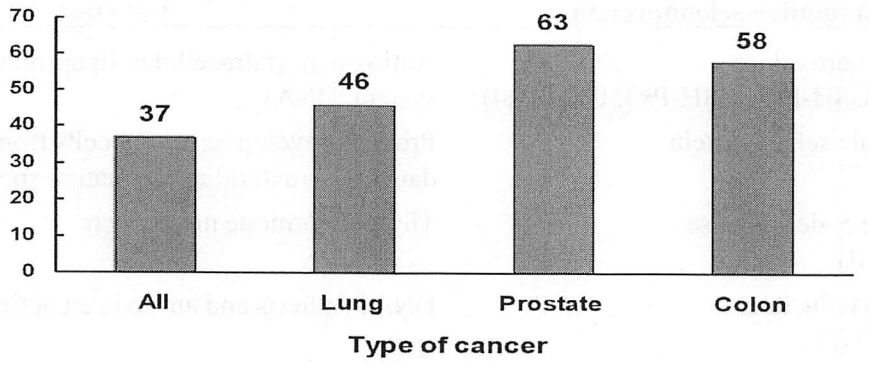
[a] British Nutr. Foundation (2001)

Individuals with an adequate Se status and then provided with supplemental Se have shown an increased immunocompetence. Proliferation of activated T-cells from supplemental Se and are thought to become more cytoxic thus helping to destroy tumor cells (Rayman, 2000). Although the plasma Se concentration in the United States is generally higher than in many parts of the world, particularly Europe, supplemental Se in the United States has also been shown to enhance the immunocompetence of humans when 200 µg Se (Na selenite) was supplemented per day. The increased production of activated T-cells appears to upregulate the enzyme selenophosphate synthetase (Table 1). Selenophosphase synthetase is necessary for the production of selenocysteine, the essential amino acid building block of the selenoproteins.

Cancer: Several studies conducted and reported prior to 2000 suggested that low Se plasma concentrations resulted in higher incidences of cancer and subsequently greater mortalities. The work of Schrauzer et al. (1977), Clark et al. (1991), Kok et al. (1987) and Combs and Gray (1998) were all "ground breaking" reports where each showed an inverse relationship between the individuals Se status and incidences of cancer. In each study those populations consuming foods of low Se content had higher cancer rates.

One study that investigated the effects of supplementing low Se areas with supplemental Se was conducted in China. Na selenite was added to the salt at 15 mg Se per kg and the study involved a total of 130,000 people (Yu et al., 1997). This area of China reportedly had a higher than normal incidence of Hepatitis B or C and a correspondingly high mortality from liver cancer. It was subsequently demonstrated that liver cancer was reduced by 35% after a 6 year period when Se fortified their salt intake, whereas the incidence was unchanged in the non supplemented group. It was, however, the published work from Clark and co workers (1996) that pushed the role of Se as a preventative for cancer to the fore front in the United States. This study was a double blinded investigation that evaluated the effects of supplementing the diet with 200 µg organic Se (i.e., selenized yeast). The study was conducted in a region where the average intake was 90 µg Se per day, a level that was somewhat low for the United States but provided adequate Se for selenoprotein synthesis. The supplemental organic Se was added on top of their normal diet. The workers examined the incidence of several types of cancers in 1312 individuals over a 10 year period. The study demonstrated that cancer mortality was reduced by approximately 50% with 63% fewer prostate cancers, 58% fewer colonic cancers, and 46% fewer lung cancers (Figure

Figure 1. The effects of supplemental selenium (200 µg per day) for a prolonged period on the incidence of several types of cancer in humans. [a]



A 50% reduction in cancer mortality with supplemental Se
Clark et al. (1996)

Table 2. Relationship of plasma Se to the number of cancer cases in humans (1983 – 1996)

Plasma Se	Cancer incidence cases, no.			
(µg/L)	No Se	+ Se	Ratio	P < .xx
< 106	56	28	2:1	.005
106 – 121	49	34	1.44:1	.40
> 121	41	45	.91:1	.99

Adapted from the review of Rayman (2000)

1). Plasma or serum Se concentration can be used as s potential screen or indicator for the probability of getting cancer. Rayman and Clark (2000) showed that plasma Se concentration from the above individuals was low and the corresponding incidence of cancer was higher (Table 2).

The mechanism of action when higher intakes are provided is perhaps beyond Se's selenoprotein role *per se*. Rayman (2000) suggested that the effect of higher Se intakes may relate more closely to Se's ability to enhance the immune response or to produce anti-tumorogenic metabolites (e.g., methyl selenol or precursors) that can disrupt tumor cell metabolism, inhibit angiogenesis, or induce apoptosis of cancer cells. A similar suggestion (Brit. Nutr. Found., 2001) indicates that chemoprevention may stimulate the activity of the immunogenic cells. Because many countries, particularly in Europe, have low dietary Se food levels and lowered blood plasma Se concentrations than in the United States, large studies are now underway in both Europe and United States to evaluate the effects of supplemental Se on cancer incidences.

Recent evidence presented by Ip et al. (2002) suggests that the monomethylselenium compound may be active in reducing the cancer incidence more effectively than other Se compounds. Their studies evaluated methylelenocysteine (MSC), a lower homolog of selenomethionine and demonstrated that it was twice as effective in reducing cancers and that tumor cells were more rapidly destroyed compared with other forms of Se. Currently, it appears that dietary Se intakes should exceed the current nutritional RDA requirement (55 µg per day for both adult

women and men). Higher Se intakes may be necessary to effectively enhance carcinogenic protection.

Mood: The brain has a high priority for the need of Se. Neurotransmitter compounds, necessary for the transmission of nerve impulses within the brain are reduced when diets are low in Se. Several conditions (epilepsy, senility, general cognitive decline, hostile behavior, etc) have been associated with a decline in Se concentration and GSH-Px activity in the brain (Weber et al. 1991, Ramackers et al., 1994), Alzheimer's patients had only 60% of the brain Se content compared to controls (Rayman, 2000).

A depletion of Se has led to depressed mood and a more hostile behavior in adults. In a series of studies where treatment groups were placed on either a low (33μg) or high (225 μg) Se intake for a prolonged time, a more cognitive group was demonstrated with less confusion and depression when the high Se diet was consumed (Figure 2). Another study demonstrated that 100 μg Se per day decreased anxiety, depression, and tiredness (Benton et al., 1991)

Other Human Conditions and Diseases Affected by Selenium

There are several conditions where a low blood Se or low antioxidant status, attributable to Se inadequate intakes may cause or exacerbate other diseases in humans and animals. The body may become more susceptible to infectious agents or immunocompetence is compromised. These diseases or conditions are:

Asthma

ALS (Lou Gehrig Disease)

Pancreatitis

Rheumatoid arthritis

Parkinsons Disease

HIV/AIDS

Cardiovascular Disease

Diabetes

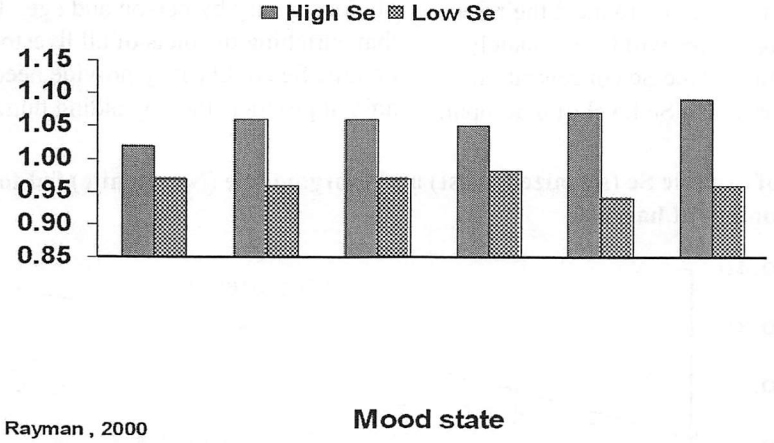
Inadequate thyroid function

Crohn's Disease

In each case of the above cases a low dietary Se intake and or a low GSH-Px production has been demonstrated. A Se deficiency can lead to the accumulation of oxygen enriched free radicals thus causing the cells to be damaged.

In general, most studies have suggested that although the RDA for Se is estimated at 55 μg Se per day for adults higher intakes seem to be necessary to

Figure 2. Influence of the selenium content of foods consumed on the mood states.



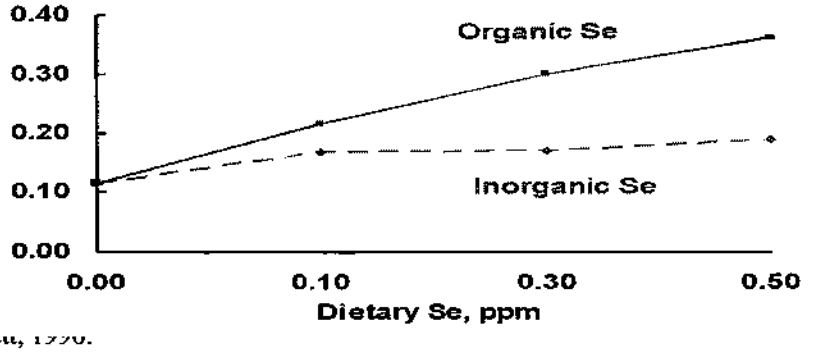
optimize human health. In many studies the addition of 100 to 200 μg per day has been found effective. The form of Se that should be consumed can be either inorganic (Na selenite) or organic (selenomethionine) but the use of organic Se is perhaps safer.

The Role of the Swine and Livestock Industries in Providing Se

Although the FDA approved the addition of inorganic (Na selenite or selenate) and organic (selenized yeast) Se to a maximum supplemental level of 0.30 ppm Se (FDA, 1987, 2002), research has been active using both forms. Both inorganic and organic Se sources maintain similar production of the essential selenoproteins. Because the organic Se yeast source contains Se within the methionine molecule (selenomethionine) this form of Se is used not only for selenoprotein production, but the Se containing amino acid can also be stored in body tissue more effectively than inorganic Se or other organic Se sources. Selenomethionine is homologous to methionine in that the pig cannot metabolically distinguish between the two homologs and uses then equally, based on their ratio in the circulatory system and the need for methionine in protein synthesis. Because methionine is an essential amino acid the pig cannot synthesize either form, and it must obtain them from the diet. Consequently, when body proteins and muscle are being produced those diets that are high in selenomethionine will produce higher Se contents in the tissues in a proportional ratio of dietary selenomethionine to methionine. When pigs are fed organic Se (i.e., high in selenomethionine) to meet their Se requirement, the selenoproteins will be adequately produced but the resulting tissue Se concentration will rise linearly to at least to a Se level of 0.50 ppm; whereas, when Na selenite is added, the rise in tissue Se is relatively low (Figure 3). Other species demonstrate the same phenomena. Figure 4 demonstrates the effectiveness of organic Se in the milk of lactating dairy cows when fed either organic Se or inorganic Se at 0.20 ppm Se. The importance of this is that Se enriched pork and other animal products may be used by humans as a source of Se and subsequently improve the Se status of the human consuming the product.

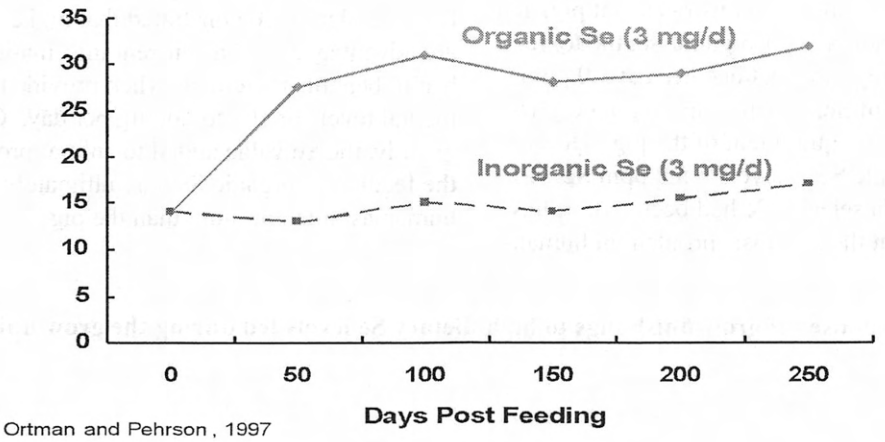
Several studies have estimated the average human Se intake in the United States. Generally, these estimates range from 79 to 104 μg per day. Over 60% of dietary Se intake is obtained from animal products (Figure 5). The Se status of individuals varies by region with those in the west central and north central areas of the United States having a high Se status because they are consuming grains and meats of higher indigenous Se (organic Se) contents. When organic Se is fed to swine and other livestock species, as compared to Na selenite, the Se content of these products are approximately doubled in their Se content. The data in Table 3 presents the amount of Se in a pork chop (i.e., 5 oz) when the animal was fed organic Se (51 μg), or when Na selenite was fed (22 μg). Similarly, a beef steak, an egg, and milk would have double the Se intake when organic Se is fed to the animal. A simple calculation demonstrates that if a person consumed 2 eggs, a glass of milk (8 oz) and 2 pork chops during the day, the Se intake would be approximately 157 μg per day compared to 74 μg per day when selenite was fed, resulting in an increase of 83 μg additional Se. Consumption quantities would obviously vary by person and age. It would appear that enriching the diets of all livestock species with organic Se could easily provide needed Se in the animal products thereby aiding human health.

Figure 3. Effect of organic Se (selenized yeast) and inorganic Se (Na selenite) fed to grow finish pigs on resulting loin Se content at harvest.



Mahan and Parett, 1996.

Figure 4. Effect of organic Se (selenized yeast) and inorganic Se (Na selenite) on the Se milk content of lactating dairy cows.



Ortman and Pehrson , 1997

Figure 5. Relative intake of Se from four food groups in the United States.

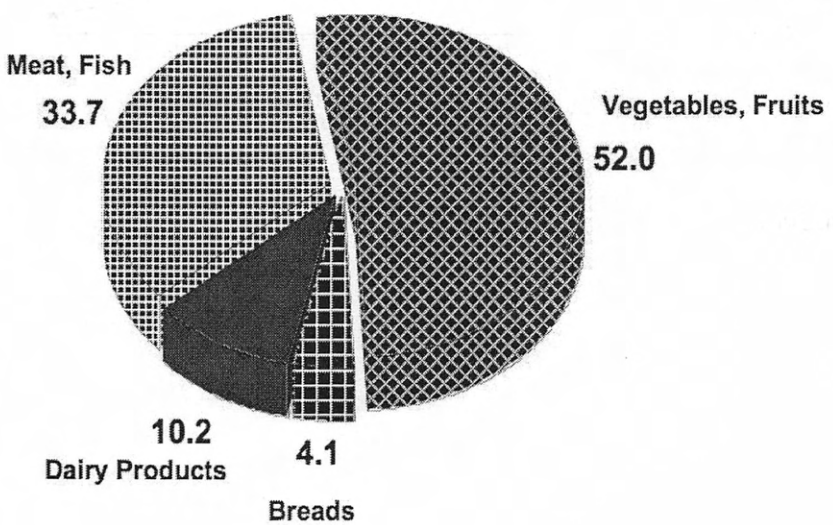


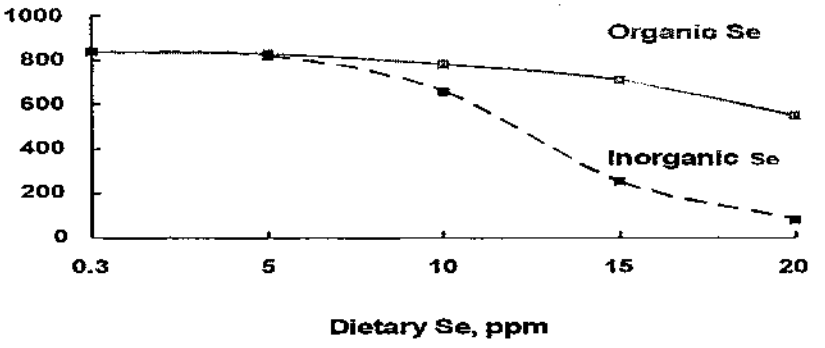
Table 3. Selenium contents in Animal Products [a]

Item	Dietary Se	Se control when fed		Serving	Se intake from tissue	
		Selenite	Organic Se[a]		Selenite	Organic
		mg/kg			mg Se/d	
Pork chop	0.30 ppm	0.15	0.35	5 oz.	22	51
Beef steak	0.30 ppm	0.13	0.26	8 oz.	30	63
Egg	0.30 ppm	0.13	0.25	2 oz.	26	50
Milk	3 mg/d	15	22	8 oz.	3.5	5

Although Se toxicity has been a concern in the past, organic Se would be safer with less problems associated with its consumption than with Na selenite. The data in figure 6 demonstrates that pigs fed various levels of inorganic or organic Se, the toxicity or selenosis symptoms that occurred at 5.0 ppm Se for either form of Se, which is approximately 15 times the dietary Se requirement of the pig. However, feeding organic Se at levels > 5.0 ppm Se was less toxic that if Na selenite Se had been fed. It has been estimated that the selenosis problem in humans may occurs when 1 mg (1000 µg) Se is consumed continuously over a long period (> 2 years).

Research needs to be continued and it remains to be more clearly demonstrated that higher Se intakes are advantageous. The current information suggests health benefits to humans when provided at supplemental levels of 100 to 200 µg per day. Consequently, the Se value added to animal products from the feeding of organic Se may ultimately benefit the human as much or more than the pig.

Figure 6. Responses of grow finish pigs to high dietary Se levels fed during the grow finish period.



Kim and Mahan, 2001

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