

Nonruminant Nutrition

Phytase, What is New and What Needs to be Done?

211 Overview of nutritional and environmental benefits of phytases. Gary L. Cromwell*, *University of Kentucky, Lexington.*

The majority of the P in cereal grains and oilseed meals is organically bound as phytic acid or phytate. This form of P is nutritionally unavailable to nonruminant animals due to the lack of phytase in their digestive tract. As a result, swine and poultry diets must be supplemented with highly available, inorganic sources of P to meet their P requirements. The poor bioavailability of P in the natural feedstuffs along with high dietary levels of supplemental P result in higher levels of fecal P compared with ruminant animals. In the early 1970s, research at Arkansas showed that supplementing a chick diet with microbial phytase improved the utilization of P. Studies in the early 1990s at Kentucky and Michigan showed that feeding pigs a low-P, corn-soy diet supplemented with phytase from a mutant strain of *Aspergillus niger* improved the bioavailability of P. At about the same time, research in the Netherlands and at our station also demonstrated the efficacy of phytase produced by recombinant *Aspergillus niger* in studies with pigs and chicks. In November, 1995, a commercial source of recombinant-produced phytase (Natuphos®) was approved for use in the USA. Since then, other sources and forms of phytase have been developed and evaluated. A considerable amount of research has been conducted with phytase. From 1992 to 2001, 82 papers involving phytase were published in Poultry Science (n=55) and the Journal of Animal Science (n=27), with 48 of these published in the last 4 years. The studies clearly show that phytase increases the digestibility and bioavailability of P from phytate, reduces the amount of inorganic P needed to maximize growth and bone mineralization, and markedly reduces fecal excretion of P. Phytase also seems to increase the bioavailability of Ca, Zn, and other divalent cations that otherwise bind to phytate. Some studies suggest that phytase may improve ileal digestibility of amino acids slightly, but other studies have not shown this response. This new technology offers substantial benefits to swine and poultry production by reducing the potential for environmental problems associated with excess P excretion.

Key Words: Phosphorus, Phytase, Phytate

212 Comparative properties of various phytase genes and proteins. E. Mullaney*, *SRR-ARS-USDA.*

Today the market for phytase as an animal feed additive is over 500 million dollars. Most of this market is for a fungal phytase that was first identified in 1968. Developments in molecular biology in the following years enabled its gene to be cloned and over-expressed. Commercialization of this enzyme has proceeded rapidly over the last decade. During that period other phytases have been characterized and several have had their genes cloned. These phytases include isolates from fungi, bacteria, and plants. Biochemical research on these different enzymes has revealed various active site motifs that are represented. These include histidine acid phosphatase, beta propeller phytase, and recently a purple acid phosphatase. The importance of disulfide bridges and the differences in the catalytic properties of fungal phytases have also been investigated. Furthermore, X-ray crystallization research of several phytases has provided details on their physical structure and how they interact with their substrate, phytic acid. Given this extensive number of studies on the properties of these phytases and the diversity of catalytic mechanisms, this information constitutes a valuable resource for further improvement of this enzyme. A review of the most recent research on the properties of these enzymes and the genes encoding them will be presented.

Key Words: Phytase, Acid phosphatase, Phytic acid

213 Expression, engineering, and testing of phytases. X. G. Lei*, *Cornell University.*

Phytases initiate the release of phosphate from phytate (*myo*-inositol hexakisphosphate), the major phosphorus (P) form in animal feeds of plant origin. These enzymes have been supplemented in diets for food animals to improve P nutrition and to reduce their P excretion. An "ideal phytase" is expected to be effective in releasing phytate-P in the digestive tract, stable to resist inactivation by heat from feed processing and storage, and cheap to produce. Site-directed mutagenesis, based on three-dimensional structure data and sequence comparisons of various

phytases, has been successfully applied to improve their pH optimum and catalytic efficiency. Different phytase expression systems, including plants, bacteria, fungi, and yeast, bear respective advantages and limitations. Effectiveness of the improved phytases should be tested with animal feeding under practical conditions.

Key Words: Phytase, Gene expression, Protein engineering

214 The EnviropigTM physiology, performance and potential contribution to nutrient management. C.W. Forsberg*, J.P. Phillips¹, S.P. Golovan¹, R.G. Meidinger¹, M. Cottrill¹, A. Ajakaiye¹, M.Z. Fan¹, D. Hilborn², and R.R. Hacker¹, ¹University of Guelph, Guelph, ON, ²Ontario Ministry of Agriculture, Food and Rural Affairs, Woodstock, ON.

The EnviropigTM produces salivary phytase encoded by the AppA phytase gene from *Escherichia coli*. This enzyme is produced by the salivary glands in sufficient quantities to allow almost complete utilization of phytate present in the diet. This results in a reduction of greater than 60% in the phosphorus content of feces from pigs receiving an industry standard diet lacking supplemental phosphate. Similar performance results have been obtained with two additional lines of transgenic phytase pigs. Phosphorus present in the feces appears to be primarily of gastrointestinal endogenous origin. Manure produced by the transgenic pigs contains a similar proportion of soluble inorganic phosphorus to total phosphorus as that in feces from non-transgenic pigs, but the content is proportionately lower. The NMAN2001 nutrient management program developed by the Ontario Ministry of Agriculture, Food and Rural Affairs for assessing maximum pig units per acre without phosphorus and nitrogen pollution from the spreading of manure was applied to the EnviropigTM. It was found that for a soil not subjected to erosion, 38% less land would be required for the spreading of manure from an EnviropigTM herd as compared to a non-transgenic herd, while in the case of a soil subjected to erosion, such as a sandy soil with a sloping terrain 63% less land would be required for the spreading of manure from an EnviropigTM herd. If crude protein in the diet was reduced with the simultaneous inclusion of limiting essential amino acids, the acreage could be reduced even further for some soil types. Because of these very optimistic results work is continuing on the characterization of the EnviropigTM.

Key Words: EnviropigTM, Phytase, Pig

215 Considerations on the field application of phytase. D. R. Cook*, *Akey, Lewisburg, OH.*

The successful application of phytase under practical feed milling and livestock production conditions is dependent on nutritional, stability, regulatory, and statutory issues, as well as ingredient availability and milling capabilities. The dietary level and phosphorus equivalency of phytase chosen for complete feeds plays a crucial role in determining economic viability of phytase use. Increased phytate phosphorus digestibility in response to graded levels of phytase follows a curvilinear pattern. Phytase levels on the linear portion of this curve will result in phosphorus release for the least cost per unit of phytase and should be considered. Whether or not phytase is given credit for improving amino acid and energy digestibility will further influence cost-effectiveness. Ingredient availability (e.g. liquid fat, meat and bone meal) and diet formulation on energy density also will influence cost effectiveness of phytase. Susceptibility of phytase to degradation from heat and humidity forces distributors, feed mills, and producers to carefully manage inventory to avoid losses in potency and necessitates a safety margin to account for product losses over normal transportation and storage times. Feed mills need to ensure tag claims match units of activity based on manufacturer definitions (e.g. FTU vs FYT vs PTU). Local and state laws regulating phosphorus application may force livestock and poultry producers to use phytase. Alternatively, some cropping systems which rely on livestock and poultry effluent may prefer higher levels of phosphorus than provided in feeds formulated with phytase. Matching phytase potency to ingredient handling and feed mixing capabilities is crucial for adequate mixing of phytase in complete feeds. Feed processing methods (e.g. expansion, pelleting) will influence at what point in the feed manufacturing process phytase is applied (e.g. added at the

mixer vs. post-pellet application). While it is clear that dietary phytase improves phytate phosphorus utilization by monogastrics, practical application of this technology for optimum efficacy and cost-effectiveness requires an integrated approach.

Key Words: Phytase, Phosphorus, Field application

216 Phytase does improve energy, protein, and amino acid utilization. Z. Mroz*, *Institute for Animal Science and Health, IDTNO Animal Nutrition, Lelystad, The Netherlands.*

Feeding phytate-rich diets with supplemental microbial phytase has often resulted in improved ADG and/or feed efficiency of pigs. This implies that (1) not only minerals but also organic compounds from intrinsic phytate complexes could be "liberated" intraluminally; (2) de novo complexing/precipitation of dietary or endogenous protein with phytic acid in digesta could be diminished or prevented; (3) the activity of endogenous enzymes could be inhibited to a lesser degree with decreasing contents of phytic acid in digesta. The extent to which dietary protein and/or energy digestibility/utilization from phytate-rich diets is improved by microbial phytase seems to be a result of such factors as (1) acidity/buffering capacity of individual ingredients, feeds, and intragastric/intraluminal contents; (2) sources and levels of dietary phytate, phytase, protein, and energy; (3) feeding regimen (restricted/ad libitum); (4) specific configurations and stability of phytate complexes; (5) the degree of synchrony of energy and nitrogen release in the small intestine with body protein/fat accretion patterns. Based on the available literature data it can be concluded for practical implications that corn- or cereal-soybean based diets supplemented with 500 FTU/kg microbial phytase (Natuphos) may have improved apparent ileal digestibility of CP/essential AA in the range from 1 to 3%-units, and DE from 32 to 46 kJ/kg of diet.

Key Words: Pigs, Microbial phytase, Protein and energy availability

Production, Management, and the Environment Environmental Stress on Livestock and Economic Implications

218 The physiological response to stress. Robert Collier*¹, Wolfram Alison¹, and Coppola Crista², ¹*University of Arizona, Department of Animal Sciences,* ²*Colorado State University.*

Stress is an external event or condition which results in a strain on a physiological system. This strain can be measured on farm by reduced productivity or animal health. Under controlled laboratory conditions the strain can be measured as increased basal metabolic rate, increased adrenal axis secretion, reduced immune function or reduced reproductive performance. In future, gene expression technology will permit identification of specific genes which are turned on or off during periods of stress. This will allow researchers to identify markers for stress at the molecular level. Major stressors in animal production systems are associated with animal handling, housing and feeding practices. High productivity itself does not constitute a stress to animals. However, failure to alter management practices to accommodate higher production does impose a stress on animals. For example, doubling of average milk yield per cow in the last 50 years has increased dairy cattle cooling requirements in summer and reduced heating requirements in winter. Failure to modify housing structures to accommodate this change results in stress on animals. It has been proposed that increasing milk production with exogenous somatotropin (bST) is stressful to cattle. However, bST does not alter basal metabolism, adrenal axis or immune function and production is increased. Increased heat production associated with increased milk yield in bST treated cattle is accommodated with a concomitant increase in evaporative heat loss via increased sweating rate. Key to reducing stress in domestic animals is not to reduce production levels but to improve animal management practices

Key Words: stress, physiology, production

217 Does supplemental dietary microbial phytase improve amino acid utilization?. O. Adeola*, *Department of Animal Sciences, Purdue University.*

Environmental concerns emanating from the excretion of large quantities of phosphorus in effluents from intensive animal production operations have led to the current routine use of microbial phytase. Following extensive investigations, microbial phytase supplementation of diet has been shown to consistently improve the utilization of phytate-bound phosphorus and a plethora of data is available in the literature to support this. The release of phosphorus from phytate during the digestion process is theorized to release other nutrients that may be bound in the phytate complex. Furthermore, hydrolysis of phytate is hypothesized to attenuate the inhibitory effect of phytate on digestive enzymes and consequently ameliorate the depression of nutrient absorption. While a limited pool of data exists on small increases in apparent amino acid digestibility in both swine and poultry literature, these have seldom translated into improved growth performance when the effect of enhance phytate-phosphorus utilization is factored out. Conversely, there are also data on a lack of response in amino acid utilization (both pre- and post-absorptive) to microbial phytase supplementation. Several factors may play important roles in amino acid utilization response to dietary microbial phytase supplementation. Identification of such factors and quantification of their effects on the magnitude of response to phytase would be important in ascribing meaningful "amino acid response factor" to dietary supplemental microbial phytase and moving the swine and poultry nutrition industry ahead.

Key Words: Swine and poultry, Phytate and phytase, Amino acid digestibility

219 Environmental stress in beef cattle. T Mader*¹, ¹*University of Nebraska.*

The performance, health, and well-being of cattle are strongly affected by climate. While new knowledge about animal responses to climatic stress continues to be developed, managing cattle to reduce the impact of adverse weather remains a challenge. During the summers of 1992, 1995, 1997, and 1999, reported feedlot death losses in the Midwest averaged between 1,000 and 5,000 head each year as a result of severe heat episodes. In the winters of 1992-93 and 1997-98, feedlot death losses exceeded 50,000 as a result of snowstorms and/or extended periods of cold, wet weather. In the winter of 1996-97 reported cattle deaths (feedlot and cow/calf) in the Northern Plains approached 250,000 head due to excessive snowfall coupled with sustained periods of sub-zero wind-chills. More recently, feedlot cost of gains averaged 12% greater for cattle finished in February, March, and April of 2001 compared with the same period in 2000 (Feedstuffs data). Individual feedlots incurred deaths approaching 1,000 animals or over five times normal death losses. In many livestock operations, alternative management strategies are needed to mitigate climatic stress in cattle. Altering the microclimate by providing protection from the environment is one of the most useful tools helping animals cope with climatic conditions. However, changes in facilities and management strategies do not need to eliminate environmental stress completely, but rather minimize the severity of the environmental challenge and aid the animal in adapting to it. Inexpensive management alternatives, such as the use of bedding in winter or sprinklers in summer, need to be considered. When designing or modifying facilities it is important that changes made to minimize impact of the environment in one season do not result in adverse effects on animals in another season. Using permanent wind barriers to minimize cold stress in the winter may require that shade or sprinklers be provided in the summer to minimize heat stress. In addition to facility changes, dietary manipulation may be beneficial for feedlot cattle challenged by environmental conditions.

Key Words: Cattle, Climatic stress, Management strategies