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ANIMAL AGRICULTURE'S FUTURE THROUGH BIOTECHNOLOGY, PART 4

BIOTECHNOLOGICAL APPROACHES TO MANURE NUTRIENT MANAGEMENT

INTRODUCTION

Food animals are fed and produced for the purpose of feeding humans. Manure from these animals is a valuable source of fertilizer, but concentrations of manure nutrients such as phosphorus (*P*)[1], nitrogen (*N*), and metals may exceed needs for plant growth and cause environmental pollution. Thus, managing livestock manure nutrients has been a task shared by those interested in animal nutrition, agronomy, and environmental protection. Many strategies can be used to monitor manure nutrient composition, and *biotechnology* has emerged as one of the most effective tools for this purpose.

Biotechnology, in this context, is regarded as methods and products derived from genetic material deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) manipulations and/or protein engi-

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neering. This type of technology may be applied to decrease total manure mass or concentrations of P, N, ammonia (NH_3), trace elements, and other factors.

Targeted modifications can be based strategically on plants, animals, ruminal and intestinal microorganisms, and diets. The plant-based approaches include genetic and chemical modifications of feeds (e.g., overexpressing hydrolytic enzymes such as *phytase* in seeds, decreasing poorly digestible components such as *phytate*, and enriching essential nutrients such as amino acid lysine). Animal-based approaches feature genetically engineering or modifying key enzymes and pathways in tissues for enhancing nutrient digestion and use. One recent success is the creation of the "Enviro Pig" that can digest feed P from phytate effectively. Microorganism-based approaches aim to modulate the metabolism of the inhabitant microbes or modify the microbial genes for improving feed fermentation and adding new digestive capacities. Nutrition- or diet-based approaches are designed to improve dietary nutrient balance and bioavailability for the physiological needs of animals.

[1]Italicized terms (except species names) are defined in the Glossary.

The primary purpose of this Issue Paper is to review the existing biotechnology (including novel technology) applied to manage animal manure nutrients. This paper differs from two related, previous CAST issue papers (IP 21, *Animal Diet Modification to Decrease the Potential for Nitrogen and Phosphorus Pollution*, and IP 23, *Biotechnology in Animal Agriculture: An Overview*) in that the current one serves as a more comprehensive and updated review of developed biotechnology—the advances in transgenic animals and microorganisms, in particular. New topics discussed include potential biotechnology derived by *genomics approaches*, integration of multiple biotechnologies in production conditions, and industrial and societal issues related to biotechnology for manure nutrient management.

NUTRIENT COMPOSITION OF ANIMAL MANURE

Manure Mass and Composition

Total collectable manure produced in the United States amounts to approximately 56 million dry metric tons per year (CAST 1996), an amount that could be used as a plant nutrient resource. The nutrient value of this quantity of manure has an economic value of $3.4 billion per year and would replace 15% of the fertilizer N and 42% of the fertilizer P purchased for crop production, respectively.

A recent publication (ASABE 2005) has detailed the average composition of freshly excreted manure by different species at different life cycles of typical livestock and poultry production. The amount and composition of freshly excreted manure can vary considerably and is influenced primarily by the original composition of the diet, species, and feeding management. Factors affecting manure composition at the time of removal and application to land include type and time in storage, and amount of added water, bedding, spilled feed, soil, and other contaminants. Climatic conditions such as temperature, humidity, and air movement can influence the rate of microbial degradation, *volatile losses*, and moisture content of the manure.

Environmental and Agricultural Impacts

Manure is an inevitable by-product of animal production, but excess manure production or nutrient excretion not only represents inefficiencies but also can cause a series of negative environmental impacts.

Water Quality

More than 56,000 kilometers (km) (35,000 miles) of rivers in the United States have been estimated to be damaged by runoff manure and excessive nutrient loading to agricultural land from animal feeding operations, with 60% of these waterways unfit for fishing or swimming (USEPA/USDA 1998). Nutrients, especially N and P, organic matter, and microbial organisms, are the primary concerns from animal manure pollution. Increased *nitrates* in water can cause methemoglobinemia, commonly called "blue baby syndrome" (Johnson and Kross 1990), although the occurrence is rare in the United States. The nitrate molecule is converted to nitrite, which replaces oxygen on hemoglobin and starves the infant for air.

Similarly, N, especially in the ammonium form, can stress aquatic life at a very low concentration and is toxic to fish at excessive levels. Elevated P concentrations in water cause algal blooms and *eutrophication* of water, resulting in decreased oxygen levels and fish kills (Correll 1998). There are approximately 18,000 km^2 of a hypoxic "dead zone" in the Gulf of Mexico, the largest water body adjacent to the United States that is so affected (CAST 1999). The hypoxic condition is derived from a long-term nutrient enrichment, primarily from organic matter (sediment) and N flow into the Mississippi Basin, and the severity of the condition varies from year to year. Such dead zones occur in water systems around the world, including several in the United States: the upper East Coast shoreline (North Carolina and Chesapeake Bay), upper Northwest shoreline (Oregon and Washington), and southern California shoreline.

Air Quality

Odorous and gaseous compounds are emitted from manure immediately after excretion because of microbial metabolism in the digestive tract of the animal. Further decomposition occurs in storage, resulting in significant gaseous emissions and odors that may impact air quality (Le et al. 2005). These emissions include nitrogenous and sulfur compounds, volatile organic compounds, and greenhouse gases (methane, carbon dioxide, nitrous oxides) that may cause acid rain (nitrous oxides, sulfates) on land and surface water. Greenhouse gas emissions can contribute to the reduction of the Earth's ozone layer. High concentrations of NH_3 emissions and gases created from the digestion of manure slurry in pit systems of confinement facilities can lead to nasal and lung irritation in workers caring for livestock in these facilities, if

buildings are poorly ventilated (Zhang et al. 1998).

Soil Quality

Excess application of manure to the soil is one of the greatest concerns regarding livestock and poultry production. This practice creates an accumulation of nutrients, with the potential impact of enriching water sources during runoff to surface water or leaching into groundwater supplies. Soil nutrient imbalances also can impair nutrient uptake and cause nutrient deficiencies in plants. The nutrients that may cause soil quality problems or threaten water quality when excess levels accumulate in the soil are primarily N and P (CAST 1996). Manure N, P, and potassium (K) composition is not properly balanced for plant uptake by typical crops grown in production agriculture. For example, pigs fed commercial diets produced manure, stored in an under-floor liquid pit, with the relative ratio of N, phosphate (P_2O_5), and potash (K_2O) of approximately 1:1:1. When based on fertilizer recommendations for N and crop removal rates for P_2O_5 and K_2O, corn grain production requires roughly a 3:1:1 ratio, and if corn is grown for silage, then approximately a 2:1:2 ratio of N, P_2O_5, and K_2O is required. Therefore, if under-floor liquid pit manure is applied to meet the N requirement of corn grain production, manure P application will be approximately 3 times crop P removal where minimal N is lost during storage and land application. Uncovered earthen pits and lagoons typically lose more N than under-floor pits, resulting in an even greater excess of manure P for crop P removal.

Potential Targets for Modifications

Decreasing the total amount of animal manure will decrease the output of all manure nutrients into the environment. Specifically, decreasing manure concentrations of P will produce a more balanced "fertilizer resource" for crops. Because manure production and excessive nutrient excretion largely result from indigestibility of nutrients in animal diets and imbalanced formulation of nutrients, enhancing the nutritional quality of feeds in diets becomes a key target for the biotechnological management of manure nutrients. This goal might be accomplished by chemically, genetically, or metabolically modifying feed and animals.

More practically, manure production and nutrient excretion can be decreased by balancing diets using synthetic nutrients such as amino acids, as well as mineral sources and exogenous enzymes, to meet specific nutrient needs of animals. Controlling emissions of manure

gaseous compounds such as NH_3, hydrogen sulfide, and volatile organic compounds will alleviate air quality concerns associated with animal production. Specific fermentable feed ingredients may be used to control manure odor and to balance manure composition for crop use. Biotechnology potentially can alter or control the biodegradation or retention of nutrients during storage of manure and after the manure is placed in the soil.

PLANT-BASED APPROACHES

Phytase Production in Plants

The manipulation of nutritional content, quality, and availability in plants has the potential to provide "designer feeds" for decreasing manure nutrient output. Managing nutrients by controlling animal diets is easier than intervening after release of these potential pollutants into the environment. For example, increasing the availability of phytate-P after ingestion by animals has been a major focus in feed crops (Raboy 2001). Phytate functions in P and mineral storage in plant seeds, but has been implicated in many other diverse cellular functions. Nonruminant animals use dietary phytate-P poorly because of inadequate amounts of the hydrolytic enzyme phytase in their digestive tracts. Because of its negative charge, phytate also hinders the availability of some essential cations such as calcium, zinc, and iron. As a result, a large portion of feed nutrients ends up in manure.

Feed supplementation with microbial phytase to decrease P excretion currently is practiced in countries where agricultural P is heavily regulated, such as The Netherlands. Because of cost considerations, however, routine phytase supplementation has not been implemented widely in locations where P reduction is not mandated. Production of a phytase in plant systems has been explored as a direct feed source for the enzyme. *Expression* of microbial phytase has been achieved in tobacco, soybean, and canola, and the recombinant enzymes have been shown to decrease P content in manure in animal feeding trials (Denbow et al. 1998; Pen et al. 1993; Ponstein et al. 2002). But plant seeds that require extensive processing before use in feed are not ideal candidates as a source of phytase, because extraction and pelleting conditions can inactivate this enzyme.

Low-Phytate Grain and Oilseeds

The decreasing of phytate content in plant seeds offers an alternative to phytase supplementation for improved P digestibility. Low-phytate lines have been generated for a number of crops, including corn, barley, rice,

and soybean (Raboy 2001). Decreases in phytate P in mutant corn lines ranged from 50 to >95%, with an accompanying increase in available phosphates. Incorporation of low-phytate corn in poultry diets led to a decrease of P content in manure ranging from 9 to 40% (Ertl, Young, and Raboy 1998) and was effective in improving nutrient use for growing pigs (Veum et al. 2001).

Soybean mutants with decreased seed phytate content also have been identified, but agronomic performance also was decreased in those lines (Hitz et al. 2002; Wilcox et al. 2000). One soybean line resulted in a 50% decrease in seed phytate accompanied by significant increases in inorganic phosphates, as well as a reduction in the undesirable carbohydrates stachyose and raffinose (Hitz et al. 2002). Although the presence of indigestible raffinose *oligosaccharides* in chicken diets has been shown to decrease true metabolizable energy, fiber digestion, and transit time (Coon et al. 1990), a comparison of diets incorporating low-oligosaccharide soybean meal versus conventional soybean meal showed no increase in nutrient digestibility (Zuo et al. 1996).

Selection and stable inheritance of a low stachyose and raffinose phenotype has been demonstrated in soybean (Sebastian et al. 2000). Soybean meal from this line resulted in a 12% increase in true metabolizable energy and gross energy use compared with conventional meal, and there was a concomitant decrease in poultry excreta produced. Another factor to consider in any phytate reduction strategy is whether there may be a trade-off between gains in nutrient management and possible beneficial effects of phytate resulting from its antioxidant properties (Graf and Eaton 1990).

Other Traits Affecting Nutrient Value of Seeds and Forage

High-oil and high-lysine corn varieties also offer improvements in nutrient output in manure. Higher oil content in grain provides more energy per unit volume of feed, enhancing animal growth performance and decreasing manure output per unit of growth. Although lower grain yields for the high-oil hybrids are still a concern to producers, new technologies for high-oil corn production have minimized yield penalties (Thomison et al. 2002). High-lysine corn can be used to better balance the essential amino acids in grain-based diets for meeting the nutrient requirement of animals and decreasing N excretion (Mertz, Bates, and Nelson 1965). The high-lysine corn is the result of a mutation in the Opaque-2 gene, which controls *zein* expression. Zeins are major

storage proteins with particularly low lysine content. Corn mutants with decreased zein content show increased levels of other storage proteins that are rich in lysine and tryptophan. The high lysine and softer kernel endosperm of the opaque-2 mutants result in a feed component that is more digestible than normal corn.

Forages are a major source of feed for livestock worldwide, especially for *ruminants*. The most important limitation to forage digestibility is the presence of *lignin* compounds in plant cell walls. Low-lignin varieties such as brown midrib corn have been available for more than 75 years, and although these mutants are highly digestible, the brown midrib trait also results in poor yields, pest resistance, and lodging. Animal performance studies with brown midrib 3 (bm3) corn also have yielded mixed results. Beneficial effects of the use of bm3 mutants on milk production in lactating cows have been observed (Oba and Allen 1999), but improved digestibility did not improve performance in beef steers (Tjardes et al. 2000). Decreasing several enzymes in lignin biosynthesis resulted in increased dry matter digestibility in forages such as alfalfa and tall fescue (Baucher et al. 1999; Chen et al. 2003, 2004; Guo et al. 2001). Furthermore, in contrast to the bm3 mutants, transgenic plants from the tall fescue study (Chen et al. 2003) showed no significant difference in a number of agronomic traits.

ANIMAL-BASED APPROACHES

Potential of Genetic Modification

Genetic modification of food animals to improve digestive efficiency has been a tantalizing objective that has had mixed success but has provided considerable basic knowledge. Because of their prolific nature, short reproductive cycle, and amenability to the introduction of novel genes, pigs have been the primary research target for growth enhancement, P use, and plant cell wall digestion. But genetic manipulation of poultry for improving P use also may become a reality.

Pursel and colleagues (1989) developed transgenic pigs with an increased level of bovine growth hormone that showed an 18% increase in feed efficiency and an 11–14% increase in growth rate. These improvements dramatically decreased manure production per unit of body weight gain, but the pigs exhibited lameness, stress susceptibility, gastric ulcers, and other health problems that negated the improvement in feed efficiency. Later, Pursel and colleagues (2004) developed transgenic pigs that expressed insulin-like growth factor I (IGF-I) to determine whether directing the expression of IGF-I spe-

cifically to *striated muscle* would enhance lean muscle growth in pigs. But they observed only a decreased daily rate of fat accretion in the transgenic pigs compared with the rate in the controls. Apparently, transgenic modification of a metabolic process with multiple effects can have unexpected and often deleterious outcomes.

Golovan and colleagues (2001) developed transgenic pigs expressing an *Escherichia coli* phytase in the salivary glands. Phytase, secreted into the saliva, mixes with the incoming food particles during chewing and hydrolyzes phytate in the acidic environment of the stomach. Because phytate is the major form of P in the diet, this process releases phosphate that is readily absorbed in the small intestine. Consequently, the dietary P requirement of the transgenic pigs can be satisfied by the cereal grain diet without inclusion of supplemental P or supplemental microbial phytase. The primary benefit is an overall decrease in P excretion by the elimination of supplemental P from the diet (Golovan et al. 2001). These transgenic pigs, like conventional pigs, excrete P in the urine that is in excess of dietary requirements. A line of these phytase pigs is currently in the fifth generation and exhibits salivary phytase activities at a similar level to that of the founding pig, and equal or similar growth and reproductive performances to the conventional pigs (Forsberg, unpublished data).

Domestic poultry expressing phytase in the gastrointestinal tract would be of considerable interest because poultry also have very low intestinal phytase activity and are unable to digest phytate. Research is in progress to develop suitable phytase *transgenes* for expression of phytase in poultry (Cho et al. 2005; Guenther et al. 2005). It is interesting to note that phytase supplementation of the ruminant diet also may enhance P digestibility even with the presence of phytase-producing ruminal bacteria (Kincaid et al. 2005). Therefore, there may be a benefit gained by introducing a phytase gene into ruminants, as well.

Endowing animals with the ability to synthesize essential amino acids in the appropriate ratios would decrease the need for protein supplements and would therefore decrease the excretion of N from nonessential amino acids present in the protein supplements provided to meet all essential amino acid requirements. Rees and Hay (1995) genetically modified *mouse 3T3 cells* by the introduction of an appropriate set of functional transgenes to enable *methionine biosynthesis*. Therefore, it may be possible to genetically modify domestic animals to attain *endogenous* synthesis of the essential amino acid methionine.

The expression of genes for the synthesis of the essential amino acids threonine and lysine in food animals would be highly beneficial; unfortunately, the development of transgenes appropriately regulated to make each synthesis pathway function in tissues will be a daunting task because threonine and lysine synthesis requires four and eight enzymes, respectively. It also is useful to mention that metabolic modifiers such as bovine growth hormone could be used to enhance endogenous metabolic capacities of animals separately or in conjunction with transgenic (exogenous)-mediated pathways or capacities.

Cereal grains and plant protein supplements contain indigestible structural carbohydrate components that are not digested by nonruminant species and are excreted in the manure. A *cellulase* and a *xylanase* have been expressed in the gastrointestinal tracts of mice by the use of several different *promoters* with the intent to improve plant cell wall digestion (Fontes et al. 1999; Zhang et al. 1999). To date, no transgenic food animal has been developed that expresses either gene. A more general challenge in the generation of transgenic animals has been the low frequency of introduced transgenes by the classic microinjection method. The recent application of *lentiviral transgenesis* in pigs has achieved a 27- to 50-fold increase in efficacy of generating transgenic animals as compared with *pronuclear microinjection*, and will help in removing a major barrier to the generation of transgenic animals. The small size of the transgene construct restricts the application of the lentiviral system, however, and further research is necessary to overcome this shortcoming (Pfeifer 2004).

Safety Assessment of Genetically Modified Animals

The general guidelines for the safety assessment of foods derived from genetically modified animals and fish have been described (UNFAO 2004) and are closely aligned with those currently applied to transgenic plants. These guidelines include molecular characterization of the transgene recovered from the transgenic animal, assessment of allergenicity and toxicity of the gene product, and comparative compositional analysis with the nontransgenic counterpart to identify any unintended effects. At present, no genetically modified animals have been approved for human food consumption anywhere in the world, and there is no food safety information available on transgenic animals, although extensive food safety data are available on transgenic plants consumed by domestic and experimental animals.

A detailed review by Flachowsky, Chesson, and Aulrich (2005) reported no significant differences in the safety and nutritional value of feedstuffs containing transgenic plants. No residues of recombinant DNA or novel proteins have been detected in any organ or tissue

samples obtained from animals fed genetically modified plants. Furthermore, no horizontal transfer of recombinant genes has been detected in the environment (Ray and Nielsen 2005). Therefore, assuming large transgenic domestic animals are healthy and meet the requirements of safety testing, there would seem to be no obvious safety issues. This assessment will have to be made on a case-by-case basis, however, and likely will depend to a great extent on the nature of the transgene construct introduced into the animal.

Microorganism-Based Approaches

Pre-gastric microbial fermentation is the key to the use of plant cell wall materials in ruminants, and this process is lacking in *simple-stomached* (nonruminant) animals. Despite the extensive use of plant material in the ruminant, digestion is incomplete (Archimede, Sauvant, and Schmidely 1997), and genetic modification of ruminal microorganisms has been spurred on to enhance plant cell wall digestion. Progress has been limited because of (1) incomplete knowledge of the microorganisms that have a role in cell wall digestion, (2) poor understanding of the enzymes that have central roles in the digestive process, (3) lack of reliable genetic transformation systems for major plant cell wall digesting bacteria, and (4) a fragmentary knowledge of the ecological factors that govern persistence of *fibrolytic* bacteria and fungi in the *rumen* (Krause et al. 2003).

Despite these challenges, genetic techniques have contributed substantially to an understanding of the role of microorganisms within the rumen and lower gastrointestinal tract, and the application of new molecular techniques will improve the ability to monitor and model microbial fermentations (McAllister et al. 2005).

Microorganisms and Their Roles in Cell Wall Digestion

The plant cell wall has a meshlike structure of long β-1,4-linked cellulose strands forming a partly crystalline structure interwoven with strands of hemicellulose composed of a xylan backbone substituted with acetyl, arabinose, and glucuronic acid residues. Each of the major groups of microorganisms in the rumen—the bacteria, fungi, and protozoa—plays a role in plant cell wall digestion within the rumen and does so by using a combination of secreted and cell-bound hydrolytic specificities (Forsberg, Forano, and Chesson 2000; Hespell, Akin, and Dehority 1997). Among these groups of organisms, the fibrolytic bacteria *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *R. albus* are considered

to be the primary plant cell wall digesters, although the contribution of each to the biodegradation process is difficult to assess.

Accessibility to the polymeric substrates constituting the cell wall matrix seems to be the rate-limiting factor (Weimer et al. 1999). Inaccessibility results from the small pore size between polymers, which is in the order of 2 to 4 nanometers and is not sufficient to allow free diffusion into the wall matrix of simple globular enzymes with masses greater than 20 kilodaltons (kDa). Furthermore, the porosity is not modified during digestion. The small pore size presents a challenge for digestion of plant cell walls by ruminal microorganisms, because practically all hydrolytic enzymes synthesized are larger than 20 kDa. Therefore, enzyme action is limited to exposed surface sites.

Forage lignin, a polymer of hydroxylated and methoxylated phenylpropane units linked by means of oxidative coupling, exerts a negative influence on digestibility by ruminal organisms and accounts for a substantial portion of the indigestible material in manure. Genetic modification of forage plants (Reddy et al. 2005) may be a better approach from which to tackle this problem than attempting to enhance the process of lignin digestion by ruminal microorganisms. This rationale is based on two facts: the major ligninolytic enzymes use oxygen—a molecule that is practically absent from the rumen—in the catalytic process, and the aromatic compounds released during digestion could have a deleterious effect on the animal.

Glycosyl Hydrolases of Ruminal Microorganisms

Numerous plant cell wall *hydrolases* of ruminal microorganisms have been isolated and characterized in their native or recombinant state (Krause et al. 2003). These hydrolases include a wide array of cellulose- and hemicellulose-degrading enzymes, and enzymes that remove substituents such as acetic acid attached to hemicellulose (Kam et al. 2005). The function of these enzymes often is enhanced by attached cellulose and xylan binding modules. The oligosaccharides released by these enzymes are hydrolyzed by a variety of β-glycosidases. Recently, the 3.6-megabase genome of the ruminal bacterium *Fibrobacter succinogenes* was sequenced and annotated (Morrison et al. 2003). Work is in progress on the genomes of *Prevotella ruminicola* and *Ruminococcus albus* at The Institute for Genomic Research (TIGR 2006), and the *R. flavefaciens* genome is being sequenced at the W. N. Keck Center for Comparative and Functional Genomics, University of Illinois (University 2006).

Knowledge of the genome sequences of these bac-

teria has revealed a wealth of hydrolase enzymes, creating the short-term challenge of identifying those that are key to polymer digestion. Knowledge of the genomes of the major species of bacteria will enable an analysis of the differences between their genomes and those of closely related species; for example, *Fibrobacter intestinalis* (Qi et al. 2005). The *F. intestinalis* genome differs from that of *F. succinogenes* in many significant ways (e.g., in the presence of numerous enzymes that could affect the genome structure and gene transfer), but *F. intestinalis* possesses a related array of plant cell wall degrading enzymes. Eventually, increased knowledge of plant cell wall digestive enzymes will facilitate improvement of forage digestion and decreased manure production.

Gene Transfer and Genetic Modification of Ruminal Bacteria

Techniques for gene transfer into and among the highly active plant cell wall degrading ruminal bacteria, *Ruminococcus* and *Fibrobacter*, have not been successful in the past; however, these techniques have been successful with *Butyrivibro fibrisolvens*, *Streptococcus bovis*, and *Prevotella* sp. (Krause et al. 2003). Recently, Klieve and colleagues (2005) identified a previously unrecognized naturally occurring DNA transfer system associated with membrane vesicles released by *Ruminococcus* spp. of ruminal origin. This discovery is a valuable development that offers a new tool for analysis and genetic modification of highly cellulolytic ruminal organisms.

An example of genetic modification to enhance cell wall digesting activity was the introduction of a xylanase gene from the anaerobic fungus *Neocallimastix patriciarum* into *B. fibrisolvens* (Gobius et al. 2002). The new xylanase produced in a pure culture enhanced plant cell wall digestion, but when the genetically modified bacterium was inoculated into the rumen of a sheep, the modified bacterium was not detectable after 28 days (Krause et al. 2001). This experiment, in conjunction with other rumen inoculation experiments, clearly demonstrates that ecological factors within the highly competitive environment of the rumen present a formidable barrier to survival and growth of genetically modified bacteria. According to Kobayashi and Yamamoto (2002), one of the factors in restricting growth of genetically modified ruminal organisms added back to the rumen is a heat-sensitive antibacterial factor, perhaps a bacteriocin-like compound that inhibits the growth of closely related species.

Researchers in the future who attempt to genetically manipulate rumen microorganisms to modify the ruminal fermentation should consider the competitive conditions within the rumen (Weimer 1998). These studies clearly document the challenges to the development of genetically modified organisms that will be competitive within the ruminal environment and that will contribute to enhanced forage digestion.

The Application of Molecular Techniques to Characterize Populations of Intestinal Organisms

In the past, the identification and characterization of the ruminal microflora have been dependent on morphological identification of the fungi and protozoa, and anaerobic cultural methods for the bacteria. These techniques are both tedious and fraught with error. The use of *16S rRNA* as a marker for microbial species has enabled a thorough and reliable dissection of the gastrointestinal microbial populations (Krause et al. 2003; McAllister et al. 2005). The recent capacity to sequence genomes of intestinal organisms including *Bacteroides thetaiotaomicron* (Xu et al. 2003), *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Prevotella ruminocola* has enhanced dramatically the opportunity to identify and study the vast array of plant cell wall digesting enzymes that have roles in nutrient digestion.

The development of DNA *microarrays* for the simultaneous identification of multiple microorganisms by extraction and analysis of a sample of DNA from an ecosystem (Krause, Smith, and McSweeney 2004) greatly facilitates the analysis of microbial population changes in the gastrointestinal tracts of both ruminant and simple-stomached animals. This technique can be applied to assess the complex population changes that occur when diets are modified, or when digestive disturbances arise because of bloat or consumption of plant toxins (Al-Khaldi et al. 2002; Wilson et al. 2002). Results can be obtained in 1 day rather than weeks. Microarray methods also can be used for studying the adaptation of individual gastrointestinal organisms to changes in the growth environment.

Metagenomics, the sequencing of the genomes of all organisms present in an environmental sample, is becoming a reality with the development of new high-throughput sequencing technology (Margulies et al. 2005). Consequently, within several years, nearly complete sequences of essentially all microorganisms within the rumen and lower gut can be expected. This knowledge will change the approach to studies of the rumen microbial ecosystem and cecum and colon fermentation dramatically.

Instead of manual enumeration of microorganisms

and biochemical analysis, the first step will be a microarray analysis to identify and quantify rapidly all organisms present, followed by a *bioinformatic* analysis of the metabolic features of the major organisms present to give a snapshot of the metabolic state of the rumen/lower gut. This analysis will enable superior monitoring of metabolic function, which undoubtedly will lead to improved design of diets and to improved control of ruminal and gut function. These improvements, in turn, will aid in achieving improved digestion of dietary components and in decreasing manure output.

NUTRITIONAL OR DIET-BASED APPROACHES

Nitrogen-Related Approaches

Efficiency of use (or lack thereof) of ingested N by animals is the determining factor regarding excretion of unused or excess N back into the environment. Biotechnology can be used in different ways in nutritional or diet-based approaches to improve N use by animals, thus minimizing N excretion in manure. For example, biotechnology offers novel tools to improve understanding of the molecular mechanisms of N metabolism in the body so that nutrient composition of the diet matches physiological capabilities and/or animal needs. Ingested feeds can be reduced to absorbable entities via chemical, physical, and enzymatic actions, and the end-products of digestion are absorbed by a number of mechanisms including those involving *transporters*.

The rate and extent of digestion and absorption are governed by the amounts and activity of hydrolase enzymes and the number of transport proteins present at any one time in an animal. These enzymes and transport proteins are either genetically programmed or metabolically regulated, and nutrients are among the most significant regulators. For example, the quality of dietary protein also influences the rate of synthesis of *proteolytic enzymes* in the pancreas (Brannon 1990), whereas the quantity of protein intake influences expression of PepT1, a peptide transporter (Chen et al. 2005) thought to be responsible for absorbing the majority of protein from the digesta in the form of dipeptides.

If the use of molecular tools can help in understanding the capacity of certain aspects of protein digestion and absorption, it may be possible to add feedstuffs (including transgenic feedstuffs) in the diet for ideal combinations of amino acids to match exactly the hydrolytic capabilities expressed in the animals. With increased knowledge about the molecular regulation of gene expression, it may be useful to include stimulators (e.g., specific nutrients or "mimics") of expression of specific genes in the diet to enhance digestibility and/or absorp-

tion of feed N.

Augmentation of digestive capacity by inclusion of exogenous *proteases* in the diet can enhance protein use and likely will become increasingly important in diet formulation (Lin et al. 1992; Odetallah et al. 2003, 2005; Williams et al. 1990). These proteases may be obtained from naturally occurring and/or genetically modified microorganisms.

The tools of biotechnology provide considerable capacity for absorptive cells in the small intestine of sheep (Chen et al. 2002a, b), chickens (Chen et al. 2005), turkeys (Van et al. 2005), and pigs (Klang et al. 2005) to transport small (di- and tri-) peptides. Theoretically, it is possible that incorporation of small peptides or partly hydrolyzed proteins, or inclusion of these instead of synthetic free amino acids in the diet, would take advantage of this important physiological ability and be beneficial for animal growth and development. Some advantage could be realized simply because the absorbable substrate is presented to the absorptive surface without the need for initial digestion. Partial hydrolysis of proteins with special cocktails of proteases designed to produce mixtures of small peptides has resulted in products that can improve animal productivity (Cahu et al. 1998, 1999; Lindemann et al. 2000; Zambonino Infante, Cahu, and Peres 1997).

Just feeding a mixture of peptides, however, does not necessarily support greater productivity. The greatest advantage would be realized if the peptides presented for absorption ultimately resulted in the optimum combination of amino acids being delivered to support growth and development. The production of specific di- and tripeptides or mixtures of specific peptides can result from using the tools of biotechnology to engineer microorganisms that produce these particular substrates or to engineer plants that produce proteins that, on hydrolysis, yield the desired end products. Thus, it is possible to anticipate the advent of nutritional and other diet-based regimens that will enhance animal performance and minimize N excretion in manure.

Phosphorus-Related Approaches

During the past two decades, biotechnology has enabled the development of highly effective phytases of fungal, bacterial, yeast, and plant (intrinsic) origins for decreasing manure P excretion in areas of intensive swine and poultry production (Lei et al. 1994; Mroz and Jongbloed 1999; Simons et al. 1990). Numerous studies have shown that this type of enzyme is capable of releasing a substantial part (up to 62%) of the phytate-bound P in grain-oilseed meal diets fed to nonruminants (Han et

al. 1997). As a consequence, the amount of supplemental inorganic P can be decreased by 50% (Gentile et al. 2003), and manure P excretion can be cut by 40-60% (Knowlton et al. 2004).

Several phytase genes have been isolated from fungi (e.g., *A. niger* var. Van Tieghem, *A. funigatus*, *Peniphora lycii*) and bacteria (e.g., *E. coli* and *Bacillus* sp.) and overexpressed in different expression systems (fungi, bacteria, yeast, and plants). Recently developed bacterial *E. coli* phytases seem to be more effective in diets for swine and poultry than the commercially available fungal phytases (Augspurger et al. 2003). The superior performance of *E. coli* phytase probably is attributed to its acidic pH optimum, high catalytic efficiency, and great resistance to pepsin (Rodriguez et al. 1999).

A major constraint for the widespread application of currently commercial phytases is their inadequate heat-stability, undergoing heat denaturation during feed pelleting or expansion (Pasamontes et al. 1997b). Naturally occurring phytases become denatured between 56 and 63°C (Lehmann et al. 2000) and could not tolerate the heat (65–95°C) generated from feed pelleting. Although postpelleting phytase inclusion and/or chemical coating of phytase (Bedford 2003) may help bypass or overcome the pelleting heat destruction of the enzyme, developing thermostable phytases is still a desirable option for the feed industry. Thus, research efforts have been focused on finding naturally occurring phytases with high heat stability or generating thermostable phytases by directed evolution or site-directed mutagenesis based on three-dimensional structures of target phytases (Joyce 2004; Pasamontes et al. 1997a, b). This endeavor has led to initial success as an "experimental consensus phytase" that has been synthesized based on the homologous sequences of 13 known fungal phytases (Lehmann et al. 2000). This synthetic phytase has higher resistance to heat inactivation than all parent fungal phytases, and it effectively releases phytate P from the corn–soy diet for weanling pigs (Gentile et al. 2003).

Synergistic interactions with other digestive enzymes or novel dietary modifiers are the emerging areas of research pertaining to dietary and/or environmental applications of phytases. There are other nutritional tactics to trim P excretion from pigs or poultry. As discussed previously, using low-phytate corn or soybean meal improves bioavailability of dietary P from approximately 20 to 75%. Feeding animals with plant feedstuffs (e.g., canola, alfalfa) containing high intrinsic phytase activity (e.g., wheat, rye, and oats) or with enhanced phytase activity by gene transfer also improves dietary P use. Appropriate feed processing such as soaking or germination

of seeds helps break down phytate P (Centeno et al. 2001).

Carbohydrate-Related Approaches

Although undigested carbohydrate in manure has less environmental impact than N and P, it comprises the largest single contributor of manure and a large portion of feed energy loss. But mature biotechnology for improving digestibility and metabolic use of the carbohydrate fraction of animal feedstuffs still is lacking; thus, the future affords great potential for further advancements. Soybean meal has a relatively high proportion of *nonstarch polysaccharides* (NSP) that are poorly digested by poultry (Leske, Zhang, and Coon 1995). Oligosaccharides such as raffinose and stachyose are largely responsible for the decreased carbohydrate digestibility (Saini 1989). For example, when 30 grams (g) of corn is fed to poultry, approximately 4 g of excreta is recovered; feeding 30 g of soybean meal results in 16 g of excreta output. Because poultry consume >50% of the processed feed soybean meal, new processing procedures or plant gene modifications that influence oligosaccharide content would be beneficial. Removal of raffinose by ethanol extraction improved metabolizable energy of soybean meal in broilers (Coon et al. 1990). Although it is much easier to supplement poultry feeds with enzymes to improve soybean carbohydrate use (McNab and Bernard 1997), the plant-based approach takes advantage of exogenous enzymes produced during germination that are capable of hydrolyzing oligosaccharides (East, Nakayama, and Parkman 1972).

Wheat and barley contain 8 to 10% NSP and pentosans and glucans, respectively, that limit carbohydrate digestibility or interfere with digestibility of other nutrients (Bedford and Morgan 1996). The presence of undigested but fermentable carbohydrates in the hindgut leads to high microbial activity that increases fecal bulk and water-holding capacity of the digesta (Cheng et al. 1987). In poultry this microbial activity leads to the problem associated with "sticky droppings," which has a direct effect on excreta output and maintenance of litter quality for floor-reared poultry. Enzymes widely used in barley- and wheat-based diets are the glycanases (xylanases and beta-glucanases) that cleave NSP. Their benefits are credited to the partial breakdown of water-soluble and viscous arabinoxylans, which inhibit nutrient digestion and absorption by raising intestinal viscosity.

Canola meal and peas are common sources of vegetable proteins in animal diets, but contain 18 and 14% NSP, respectively (as cellulose and pectic polysaccharides). Pea starch and protein are located within the cell matrix, and the complete nature of cell wall structure

makes these nutrients less accessible to endogenous enzymes for poultry (Longstaff and McNab 1987). No conclusive effects of supplemental carbohydrase enzymes on growth performance in pea-fed broilers were observed (Daveby, Razdan, and Aman 1998), but enzyme supplementation proved beneficial in improving NSP digestibility of canola meal (Kocher et al. 2000).

Improvements in NSP use are achievable using combinations of *carbohydrases* that differ in substrate preference and mode of action. Nonstarch polysaccharide-degrading carbohydrases act by degrading high molecular weight polysaccharides to simple sugars, oligosaccharides, and low molecular weight polysaccharides (Meng et al. 2005). The use of pure and specific substrates might lead to false conclusions, whereas carbohydrase preparations used in combinations generally are more effective in the degradation of cell wall polysaccharides.

Unfortunately, information on the use of enzyme combinations in practical broiler diets is limited. This limitation is not unexpected, however, because most enzyme sources used in animal feeds are derived from crude products (Bedford and Classen 1993) and the microorganisms selected for enzyme production are capable of producing multiple activities (Bhat and Hazelwood 2001). In this context, the biotechnological development and use of multicarbohydrase preparations to target NSP fractions provides the greatest potential for future improvements in the nutritive value of cereal cultivars. Because a larger proportion of beta-glucans and pentosans are degraded in the small intestine (ileum) of swine by endogenous enzymes or intestinal bacteria, the improvement in performance in swine is less than that seen in poultry (Thacker 2000).

Poor use of fiber can impact animal performance and result in increased fecal output. Sutton and colleagues (1998), however, reported that adding low levels of an indigestible oligosaccharide and fibrous materials such as cellulose decreased the N content of fresh swine manure, especially the NH_3-N fraction. The pigs had an increased fermentative capacity in their cecum and colon because of resident microflora. An additional benefit from addition of cellulose to the pigs' diet was that pH of fresh and stored manure was decreased, which aids in decreasing NH_3 volatilization.

Several studies have shown the potential positive effects of supplemental enzymes, including xylanase, cellulase, carboxymethylcellulase, β-glucanase, and amylase, in feedlot diets (Beauchemin et al. 1997; Hristov, McAllister, and Cheng 2000). For dairy cattle, supplemental enzymes represent one option for improving dietary fiber disappearance rates (Kung et al. 2000; Rode, Yang, and Beauchemin 1999). A daily milk production increase of 2.5 kilograms (kg) occurred when lactating cattle were fed a total mixed diet treated with a fibrolytic enzyme product (Stokes and Zeng 1995). Results clearly demonstrate that deficiencies in fibrolytic capacity associated with low fiber digestion can be overcome partly by enzyme supplementation.

The agricultural industry constantly strives to lower the cost of animal production while minimizing the extent of environmental impact. Carbohydrates are involved in virtually every aspect of biological systems; finding ways to improve carbohydrate use from major animal feedstuffs derived from soybean, canola, corn, wheat, barley, rye, peas, and to improve the fibrous fractions of animal feedstuffs is a complex challenge. Multifaceted mechanisms are implicated, and future research will target improved seed stock development and dietary enzyme use as economical and practical methods of treatment.

Novel Dietary Modifiers

Because of impending regulations by the European Union against the use of antibiotic growth promoters in feeds for swine and poultry, there has been increasing interest in biotechnology-derived dietary modifiers including acidifiers (organic/inorganic acids); probiotics (viable, beneficial bacteria competing with pathogens); prebiotics (inulin, fructooligosaccharides, galactooligosaccharides, or mannanoligosaccharides); symbiotics; postfermentation products; antimicrobial peptides; and/or herbal extracts (Bastiaanse 2005). Most of these modifiers are supplemented to diets for nonruminants to improve their feed intake; to increase their resistance to pathogenic colonization proliferation of selected groups (more beneficial) of the gastric, intestinal, and colonic microbiota; to improve their gut development and permeability; and to enhance gastrointestinal juice secretions and/or mucosal/systemic immunity. Moreover, they also improve overall feed use and thereby contribute to managing manure nutrients.

Early studies of the complex microbiota (> 400 species) in nonruminants were limited, at least partly because only a fraction of the microorganisms are culturable with conventional methods. But systematic bacteriology has undergone a revolution in recent years with the advent of 16S rRNA sequence analysis (Collins and Gibson 1999). The 16S rRNA is an immensely powerful molecular chronometer, and for the first time has permitted the construction of an all-embracing phylogenetic, evolutionary framework for bacteria ranging from kingdoms and major phylogenetic domains to individual species. In addition, the rapid accumulation of gene sequence data

is revolutionizing the perception of bacterial diversity and the discovery of new taxa. Furthermore, 16S rRNA sequence data provide information essential to the development of molecular-based tests for identifying specific bacterial populations directly, in their natural environment, without the need for cultivation.

Biotechnological methods also have been applied for commercial production of fermented feeds (liquid/dried) and preparations of carboxylic acids and other products (Brooks, Beal, and Niven 2001; Mroz et al. 2005). Carboxylic acids, in conjunction with intraluminally generated lactic acid and volatile fatty acids, serve as natural, selective bactericides/ bacteriostatics and affect mucosal growth and systemic energy/acid-base metabolism. Benzoic acid and its salts have been considered eco-friendly compounds, because they can diminish NH_3 emission from manure by up to 44% (Mroz et al. 2000, 2005). Overall, the economic feasibility of the use of any novel dietary modifier is heavily dependent on farm structure and the restrictions and costs imposed by regional environmental and health safety policies. Hopefully, new technologies will provide "ideal" dietary modifiers that can meet both animal health and environmental needs.

MODELING THE POTENTIAL BENEFITS OF BIOTECHNOLOGY

Modeling the Effects of New Biotechnologies on Nutrient Use

Nutrition models are used in computer programs to predict nutrient requirements of animals and feed nutrient values accurately in each production situation to formulate diets that will optimize production while minimizing nutrients in manure (Harpster and Fox 2002). Thus, the practical application of biotechnologies for decreasing manure nutrients requires accounting for their effects within nutrition model equations that predict (1) animal energy and amino acid requirements for the target performance and/or (2) the digestion and metabolism of carbohydrates, protein, lipid, vitamins, and minerals in feeds.

In addition, nutrition models for ruminants need to account for the effects of biotechnologies on rumen fermentation, including microbial growth and their rate of fermentation of sugars, starches, and fibers, and on forage amount in the diet needed to maintain an optimum rumen motility, pH, and health. The following are specific examples of how nutrition models can account for expected alterations in dietary profiles provided by several of the biotechnologies discussed in this paper as well as what inputs are needed.

High-Lysine and High-Oil Corn

The purpose of using high-lysine corn in a diet is to meet essential amino acid requirements with less supplemental protein, which can decrease cost as well as excreted N. Lysine often is the first limiting amino acid in diets for nonruminants (NRC 1998) and for ruminants when the diets are based on conventional corn and by-products from corn (NRC 2001). The nutrition model used to formulate the diet must have the complete nutrient content of the high-lysine corn, especially the amino acid profile in the corn protein. Then the nutrition model uses this information to determine whether supplemental sources of amino acids can be decreased in the diet in meeting the lysine requirement.

The expected benefit of substituting high-oil corn for normal corn in the diet is to increase diet energy density, therefore decreasing the feed required for the same or improved performance. Compared with conventional corn, high-oil corn has a 4% higher energy density because it has more than twice as much fat; it also is 1–2 percentage units higher in protein (Dado 1999). But when fed to ruminants, the rumen-degradable starch will decrease, which also will decrease the amount of microbial protein produced from the corn. A nutrition model can account for these interacting effects by predicting metabolizable energy derived from both the fat and starch in the diet, and in ruminants the microbial amino acids that will be produced (Fox et al. 2004; Moate et al. 2004; NRC 2001). The information required to do this accounting includes complete feed composition information, especially the fat content and fatty acid profile of the fat in the feed, and the amino acid profile in the protein.

Low-Lignin Forage

The expected benefit of using low-lignin forage is the ability to include a higher percentage of forage in the diet of ruminants without decreasing their performance, resulting from an increased rate and extent of fiber digestion in the rumen. But inclusion of low-lignin forage in diets for ruminants fed high-energy rations creates difficulties in meeting fiber requirements, because lignin stimulates chewing and rumination. A nutrition model can account for these effects and interactions when complete information on carbohydrate and protein fractions and their digestion rates in the low-lignin forage is provided (Aquino et al. 2003). The model then can predict the impact of this biotechnology on digestibility of the forage plus the change in end products of fermentation, concentrates needed in the diet, and nutrients excreted.

Genetically Modified Microorganisms

The expected outcome of applying transgenic technology would be to increase populations of rumen bacteria that could improve the extent of starch and fiber digestion in the rumen. A mechanistic rumen model is needed to account for these effects (Nagorcka, Gordon, and Dynes 2000). Various applications of genetically modified organisms to ruminant nutrition currently are being investigated.

Example of Modeling Biotechnologies for Decreasing Manure Nutrients

The paper by Tedeschi, Fox, and Tylutki (2003) exemplifies how a specific biotechnology can be modeled for decreasing manure nutrients in a nutrition model. Published data were used to develop the following coefficients to account for the effects of the rumen fermentation modifier monensin fed at diet concentrations of 28 to 33 milligrams (mg)/kg in ruminant nutrition models similar to those described by the NRC (2000) and Fox and colleagues (2004): (1) predicted dry matter intake is decreased 4%, (2) diet net energy for maintenance used to compute feed required for maintenance is increased 12%, and (3) peptide uptake rate by rumen bacteria is decreased one-third. Then the nutrition model predicts the concentration of nutrients needed in the diet to meet target animal production levels and N and P excreted when monensin is included.

A representative diet and the number and average days fed for beef cattle finished in feedlots were used to predict the impact on N excreted by including monensin in the diets of feedlot cattle, using the Cornell Net Carbohydrate and Protein System nutrition model (Fox et al. 2004). The model predicted that N excretion is decreased by 378 g per average feedlot animal when monensin is added to the diet. With an average of 26 million steers and heifers fed annually in commercial feedlots in the United States, the inclusion of monensin in all feedlot diets was then predicted to decrease N excretion by 9,828 metric tons/year. When similar adjustment factors were developed and applied for lactating dairy cows, the impact of feeding monensin to all dairy cows in the United States was estimated to be a decrease in N excretion of 67,000 metric tons. The accuracy of these models needs validating with actual experimental or field data.

Industrial, Societal, and Governmental Issues

The term "biotechnology" brings forth a range of emotions, within which lies a reality that ultimately determines the success or failure of current and future biotechnology. Because of population growth and the increasing demand for animal foods (UNFAO 2000), global livestock production will continue to evolve from smaller family support systems to larger, more market-oriented, integrated production systems. Through this progression, issues involving environmental contamination, air quality, and animal welfare will be more commonplace, resulting in more complex relationships among animal industry, society, and governmental agencies. Because biotechnology potentially can provide important solutions to these problems, it is critical that we understand and address the associated industrial, societal, and governmental issues.

Industrial Issues

In today's livestock production environment the decision to implement new technology is driven largely by economics. Technologies not having an easily demonstrated high return on investment typically are not adopted unless other significant factors (e.g., government regulations) influence the decision-making process. For example, enzyme technologies such as phytase have been available for years but adoption has taken considerable time. Initial usage was limited to production systems located in close proximity to towns, neighbors, or special interest groups that were inclined to issue complaints or where there was strict local control of land application of manure. With time, increasing state and local regulation concerning P land application along with decreasing phytase cost have resulted in an increased rate of adoption of phytase technology.

Like phytase, other biotechnologies such as transgenic animals, enzymes, genetically modified crops, and antibiotic alternatives need to demonstrate significant added value in terms of economics, regulatory compliance, or good neighbor policies before they will be incorporated as standard management practices. In addition, for new biotechnologies to achieve broad-based application they will need to be easy to understand, simple to apply, and safe to use in terms of application, residues, and disposal. New biotechnologies also must result in an end product of acceptable quality, as defined by the consumer. Technologies that are easily understood, such as feeding technologies, will be adopted more readily than those of a more "sophisticated" nature, such as genomic modification. Industry and society must perceive agricultural biotechnology as a positive environmental technology for it to be implemented successfully (Kershen 2002). Ultimately, these technologies must exist in harmony with social ethics or the agribusiness industry will

be at risk of losing its autonomy (Rollin 2004).

Societal Issues

Currently, there seem to be conflicting views of biotechnology between society and scientists (Hodges 2003). Whereas scientists may tend to see biotechnology as a natural process in understanding nature to control or modify it, society often questions the need for biotechnology unless (1) a convincing argument can be made for solving important social issues associated with livestock production and (2) it can be demonstrated to be inherently safe. Biotechnology related to manure nutrient management will be more acceptable to society than that directed solely at improving production efficiency (Bruhn 2002; Hodges 2002). Advancements in manure nutrient management that result in improved air quality, lower production of greenhouse gases, decreased odor, and a decrease in the potential for water pollution will address concerns that are of primary interest to the public and that, as such, will gain public support more readily. In addition, the public generally will support biotechnology efforts that result in air quality improvements within the production environment (livestock housing) because these improvements will enhance animal welfare, health, and well-being.

But some biotechnologies, such as production of transgenic or other genetically modified animals, may receive additional ethical scrutiny from the public because of concerns for the health and well-being of animals. Because it is human nature to be skeptical of technology that is poorly understood or perceived to possess inherent danger, genomic technologies—whether animal-, plant-, or microbial-based technologies—may not be accepted readily by the public. It is essential that the scientific community understands the societal issues that relate to biotechnology and addresses those concerns adequately. New biotechnologies of this nature should be introduced only after thorough, independent studies that elucidate the long-term effects and that address consumers' concerns along with production efficiency and benefits.

It also is imperative that consumers be informed about the benefits that biotechnology brings to the environment, air quality, and animal well-being. The possibility of any adverse effects, whether real or perceived to be real, must be addressed. Failure to recognize and comply with the associated societal issues could result in excellent new technologies that fail to gain acceptance.

Governmental Issues

Governmental agencies play a critical role in the development and application of new technologies. Around the world, regulatory requirements designed to control the adverse effects of animal production to the environment, to improve air quality, and to decrease odor are being implemented. Permits and nutrient management plans now are commonplace, and additional requirements that restrict manure land application are gaining global support. In certain locations, nutrient and/or feed ingredient use is restricted in an effort to protect the environment. An effective example is found in The Netherlands where a Mineral Accounting System (Minas) was implemented (Ministry 2001). In other locations throughout the world, new air quality standards currently are being debated, studied, and adopted. These evolving regulatory requirements increase demand on livestock production systems to seek new technologies that are effective, socially acceptable, and economically viable. Government plays a key role in ensuring a regulatory system that is open, transparent, and effective and that permits harmonious development of animal production to maximize production while minimizing ecological risk (UNFAO 2000).

Government also should play a key role in informing the public of the benefits and risks of new biotechnologies and their function in the management of animal resources. This responsibility extends to the development of national and global strategies to address public awareness, education, and information. Government must maintain a careful balance between societal concerns, special interest influences, production efficiency, and environmental stability.

SUMMARY AND RECOMMENDATIONS

It has become increasingly important to decrease the total amount of animal manure and concentrations of specific nutrients such as P and N in livestock manure. Biotechnology has helped those in academia and industry to develop plant-, animal-, microbe-, and diet-based approaches to alleviate potential adverse effects of excessive manure nutrients on water, air, and soil quality for human living and for agriculture. Most likely, new biotechnology will continue to be generated and applied to manage animal manure nutrients. The following recommendations provide a limited list of future potentials and directions.

1. Combine a variety of nutritional enhancements in plant seeds that will offer the ability to tailor animal diets for better nutrient management. Metabolic and nutritional engineering using recombinant DNA, genomics, and model system approaches may be used for future crop improvement in nutrient

content and availability.

2. Determine the limiting enzymes in plant cell wall digestion to serve as the basis for enhancing ruminal digestion by diet design, genetic modification of forage plants, and genetic modification of ruminal organisms.

3. Develop microarray systems for rapid analysis of the ruminal ecosystem and to assist in monitoring digestive processes through the use of metagenomic analysis.

4. Define accurately the nutrient requirements of animals and elucidate the molecular mechanisms underlying the limiting steps of feed nutrient use by animals so that diet formulations match the exact physiological capabilities of animals, minimizing manure nutrient excretion.

5. Develop species- and physiological stage-specific hydrolytic enzymes as animal feed supplements to help maximize the efficacy of exogenous enzymes in diets. In ruminants, further research is required to determine the need for dietary enzymes, their effect on ruminal fermentation, and the role that protein and roughage source relative to fiber type and content might play in response to enzyme supplementation.

6. Develop inert feed additives and/or transgene technology in food-producing animals that target specific genetic capacities to optimize or introduce endogenous metabolic pathways to improve their feed nutrient use, without negative impact on the animals or the environment.

7. Use modeling to predict the impact of new biotechnology and to help its integration with other technologies for manure nutrient management.

8. Develop separate/joint educational modules about the biology behind, and the ethics of, biotechnology for manure nutrient management, and promote a balanced view of animal agriculture, manure production, environment protection, and biotechnology application.

Biotechnology will provide solutions to manure management issues that may or may not be adopted readily by industry or society. The acceptance and subsequent adoption of new biotechnology will be highly dependent on the harmonization of product benefits and effectiveness, cost, societal acceptance, and government intervention. Research and education must go hand-in-hand, because effective, ongoing education should be recognized as a critical element. All these factors must be considered and addressed for successful implementation of biotechnology in manure nutrient management.

GLOSSARY

16S rRNA. A unique single-stranded nucleic acid similar to DNA but having ribose sugar rather than deoxyribose sugar and uracil rather than thymine as one of the pyrimidine bases that has been used for the speciation of bacteria.

Bioinformatics. Mathematical, statistical, and computing methods that attempt to solve biological problems using DNA, RNA, and amino acid sequences and related information.

Biotechnology. Technical know-how, including materials and methods, related to genetic manipulations of DNA and RNA or modification and engineering of proteins for target functions or pathways.

Carbohydrases. Enzymes that catalyze hydrolysis or metabolism of compounds consisting of simple or complex sugars.

Cellulase. An enzyme/family of enzymes that hydrolyze the β(1-4) linkages of the polyglucose polymer cellulose.

Endogenous. Produced or synthesized within the organism or system.

Eutrophication. The process by which a body of water becomes either naturally or by pollution rich in dissolved nutrients (as phosphates) and often shallow with a seasonal deficiency in dissolved oxygen.

Expression. The process by which a gene's coded information in DNA is converted into RNA that usually leads to synthesis of a protein.

Fibrolytic. The process of degrading fiber (carbohydrate complex in feeds).

Genomics approach. Use of all nucleotide sequences, including structural genes, regulatory sequences, and noncoding DNA segments, in the chromosomes of an organism.

Hydrolases. Groups of enzyme proteins that catalyze the breakdown of complex nutrients such as lipids, proteins, and carbohydrates by the addition of water.

Lentiviral transgenesis. A virus-based system for introduction of a transgene into the genome of animals. In pigs the system is 27- to 50-fold more efficient than DNA microinjection.

Lignin. Complex networks of aromatic compounds called phenylpropanoids that are found in secondary cell walls of plants, accounting for a much as 20 to 30% of plant tissue.

Metagenomics. The functional and sequence-based analysis of the collective microbial genomes contained in an environmental sample.

Methionine biosynthesis. The cellular process to produce one of the essential amino acids (basic molecular unit of proteins).

Microarrays. Sets of miniaturized chemical reaction areas that may be used to test DNA fragments and gene expression.

Mouse 3T3 cells. Mouse fibroblast cells grown in cell culture.

N. The atomic symbol for nitrogen, a gaseous element with the atomic number 7 and an atomic weight of 14.

Nitrates. Salts or ester of nitric acid or compounds containing the NO_3^- group.

Nonstarch polysaccharides. Long chain of carbohydrate consisting of many simple sugars that are soluble and insoluble forms of dietary fiber.

Oligosaccharides. Short carbohydrate chains typically consisting of three to six simple sugars.

P. The atomic symbol for the widely occurring non-metal element phosphorus, with the atomic number 15 and an atomic weight of 31.

Phytases. A class of enzymes that degrade phytate by the removal of phosphate groups rendering P more bioavailable.

Phytate. The major storage form of P found in plant seeds; the chemical form is myo-inositol hexakisphosphate salt or $InsP_6$; also interchangeably called with its free acid form known as phytic acid.

Promoters. Regions of DNA to which RNA polymerase binds before initiating the synthesis of RNA based on the corresponding DNA (gene) sequence.

Pronuclear microinjection. Mechanical injection of multiple copies of a transgene into the pronucleus of a fertilized zygote, which then is introduced into the reproductive tract of a recipient female and taken to term. Offspring are tested for the presence of the transgene integrated into one of the chromosomes. The method is very inefficient because only a small fraction (~2%) of the treated embryos develop into transgenic animals.

Proteases. A subset of hydrolytic enzymes, including proteinases and peptidases, that break down of proteins or peptides.

Proteolytic enzymes. Any enzyme that catalyzes the splitting of proteins into smaller peptide fractions and their constituent amino acids by a process known as proteolysis.

Rumen. The first division of the stomach of a ruminant animal in which most food collects immediately after being swallowed , and from which it is later returned to the mouth as cud for thorough chewing.

Ruminant. Any of various hoofed, even-toed, usually horned mammals characteristically having a stomach divided into four compartments and chewing a cud consisting of regurgitated, partly digested food.

Simple-stomached (non-ruminant). Having a stomach with only a single compartment, and with limited microbial fermentation capacity.

Striated muscle. Muscle in which the repeating units of the contractile myofibrils are arranged in registry throughout the cell, resulting in transverse or oblique striations; for example, the voluntary (skeletal) muscle of vertebrates.

Transgene. Novel genes with appropriate sequences to permit expression of an endogenous protein when integrated into the host genome.

Transporters. Proteins that span and carry specific nutrients across cell membranes.

Volatile losses. The loss of substances by vaporization, typically at relatively low temperatures.

Xylanase. An enzyme that acts to hydrolase the β(1-4) linkage of the polyxylose polymer xylan.

Zein. A class of storage proteins found in corn kernels with a low-lysine amino acid content.

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