The Use of Steered Ileo-cecal Valve Cannulated Pigs to Evaluate the Effects of Adding Phytase or β-mannanase to the Diet on Amino Acid, Mineral and Energy Utilization

by

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Dedicated to the late Dr. E. T. Kornegay

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(ABSTRACT)

Forty-six barrows fitted with steered ileo-cecal valve cannulas were used in four experiments to evaluate the effects of supplementing swine diets with microbial phytase or β-mannanase on the apparent ileal (AID) and/or apparent total tract digestibility (ATTD) of amino acids, N, Ca, P, DM and energy. In Exp. 1, the addition of phytase to low CP corn-soybean meal based diets increased the AID of Ca ($P < .01$), P ($P < .001$), and all amino acids ($P < .10$) measured except Leu, Ser, Pro, Met, His and Tyr. In Exp. 2, the addition of microbial phytase to corn-soybean meal, corn-soybean meal-wheat middlings, or corn-soybean meal-meat and bone meal based diets resulted in increased AID of Ca and P, but had no effect ($P > .1$) on amino acid digestibilities. Diet type affected all digestibility measurements, but did not affect the efficacy of supplemental phytase. In Exp. 3, the addition of microbial phytase to corn-wheat-soybean meal, corn-wheat-cannola, or sorghum-corn-soybean meal based diets led to an increased ($P <.05$) AID of P, Asp, Thr, Ser, Ala, Tyr, Phe, Lys and Arg. In Exp. 4, the addition of β-mannanase to corn-soybean meal based swine diets led to an increased AID of DM and ATTD of energy. In addition, the AID of all amino acids measured were increased numerically, with many of these values approaching significance. The results of these studies demonstrate that supplementing pig diets with phytase or β-mannanase, results in an increased digestibility of certain dietary components due to the breakdown of anti-nutritive compounds in the diet.

Key Words: Pig, Phytase, β-Mannanase, Cannula, Amino acids, Digestibility

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LIST OF ABREVIATIONS

ADG	Average daily gain
AID	Apparent ileal digestibility
Ala	Alanine
Arg	Arginine
Asp	Aspartic acid
ATTD	Apparent total tract digestibility
bP	Base pair
Ca	Calcium
CP	Crude protein
Cys	Cysteine
DM	Dry matter
Glu	Glutamic acid
Gly	Glycine
His	Histidine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine
P	Phosphorus
Phe	Phenylalanine
Pro	Proline
Ser	Serine
SICV	Steered ileo-cecal valve
Tau	Taurine
Thr	Threonine
Tyr	Tyrosine
Val	Valine

Chapter I

Introduction

Historically, swine nutritionists have attempted to maximize growth rate while minimizing diet cost. The overall goal was to formulate a diet that resulted in lean tissue accretion in the animal at the least cost to the producer. More recently, concerns over the possible environmental impact of large-scale animal production operations has caused nutritionists to focus attention in a new area referred to as "environmental nutrition." Concern over the environmental impact of agriculture has stemmed from two basic areas. First, the public has become more aware that everything that is done in food production impacts the environment. Increased public awareness has resulted from better education, and a booming U. S. economy without any major military threats. Second, the number of swine farmers in the U. S. has been rapidly declining over the last 50 yr, while the number of animals raised per farm has been steadily increasing, resulting in more intensified swine operations. With intensified agriculture comes a greater manure production per unit of space. This leads to greater environmental threats due to limitations in shipping distances of manure. The outcome of increasing concerns over the environmental impact of swine operations has yet to be fully realized. There is a trend toward increased legislation to regulate land application of swine manure. This has led nutritionists to search for ways to maximize nutrient utilization from the diet, thereby minimizing nutrient excretion.

In an ideal world, the pig would utilize 100% of the dietary nutrients consumed. This, of course, is not possible due to the need to excrete by-products of metabolism to prevent toxicity, and due to the cost of creating such a diet. So, the main thrust in swine nutrition today is to maximize dietary nutrient utilization. This process is confounded by the fact that any method that increases dietary utilization must

be cost effective. Therefore, nutritionists are striving to optimize dietary nutrient utilization, by balancing nutrient retention against animal performance and cost.

Nutrient utilization by the pig is limited by the biological availability of each individual nutrient. The availability of a given nutrient varies from one feedstuff to another, and will be altered by other constituents in the diet. Therefore, the availability of a given nutrient in the diet may be increased by dietary additives which help convert the nutrient to a more utilizable form, or by adding something to the diet which counteracts the anti-nutritional effects of other dietary components. One of the newest tools nutritionists have to increase nutrient availability of the diet are enzyme preparations which can be added to the diet.

An enzyme is defined as: "a protein molecule produced by living organisms that catalyzes chemical reactions of other substances without itself being destroyed or altered upon completion of the reactions" (On-line Medical Dictionary, 1997). Enzyme supplementation to feed has been limited until recently due to the cost of enzyme production and purification. However, with advances in biotechnology, and in microbial culturing systems, it is now possible to produce enzymes at a cost that allows for their addition to swine diets. As a result, the feed industry has been flooded in the last few years with enzyme additives and other addititives, all of which are claimed to to increase digestibility and performance. The nutritionist should evaluate these products and decide if they are cost effective to add to the diet.

The broad scope of this dissertation is environmental nutrition. Specifically, the addition of phytase or β-mannanase (Hemicell) to swine diets were evaluated. The major objectives of this research were:

1) Develop and improve the steered ileo-cecal valve cannulation techniques for use in swine nutrition research.
2) Evaluate the effects of supplementing corn-soybean meal based swine diets with microbial phytase on the apparent ileal and/or apparent total tract digestibility of amino acids, N, Ca, P, DM, and energy.
3) Determine equivalency values of phytase for amino acids in corn-soybean meal based diets.
4) Evaluate the effects of supplemental phytase in various diet types on the apparent ileal and/or apparent total tract digestibility of amino acids, N, Ca, P, DM, and energy.
5) Evaluate the effects of supplementing swine diets with Hemicell on the apparent ileal and/or apparent total tract digestibility of amino acids, N, Ca, P, DM, and energy.

Literature Cited

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Chapter II

Literature Review

The literature review that follows will start by discussing the properties of P, Ca and amino acids in nutrition. The review then proceeds into a discussion of phytate followed by a discussion of phytase and its effects on mineral, amino acid, and carbohydrate digestibility. A similar section will follow discussing Hemicell which is a β-mannanase. Finally, the literature review will conclude with a discussion of ileal digesta collection techniques.

Phosphorus

Phosphorus is an essential nutrient in swine diets, serving important functions as part of structural compounds in bone and in cell membranes, as a source of high energy bonds in nucleotides, as a structural component of nucleic acids, as a component of many enzyme cofactors, and as a component in many metabolic pathways (Jongbloed, 1987; Ziegler and Filer, 1996; Berner 1997). Phosphorus has an atomic number of 15 and an atomic weight of 30.97. It can exist in a trivalent or pentavalent form. However, P, is commonly found in the body as phosphate (PO_4^{-3}) with a valence of five.

In humans, P in the body accounts for about 1% of the total body weight (on a wet basis). Of the total body P, approximately 85% is found in bone, 14% in soft tissue and muscle, and 1% in blood (Berner, 1997). Phosphorus is stored in bone as a crystalline structure, along with Ca, known as hydroxyappetite ($Ca_{10}(PO_4)_6(OH)_2$). Deficiencies in bone P lead to rickets and osteomalacia.

Phosphorus absorption occurs throughout the small intestine with the the largest portion of P absorption occurring in the jejunum (Korwaski and Schachter, 1969; Walling, 1977). There are two primary mechanisms involved in phosphate absorption, an active transport system and a passive transport system. Active transport of PO_4 occurs primarily in the proximal small intestine and it is related linearly to the lumenal Na^+ concentration (Danisi and Straub, 1980). It is blocked by calcitonin (Caniggia et al., 1968; Juan et al., 1976), while $1,25(OH)_2$-D3 has a stimulatory effect on phosphate absorption (Danisi and Straub, 1980). Passive transport occurs primarily in the jejunum and ileum and is related to the lumenal concentration of PO_4 (Danisi and Straub, 1980). Therefore, when the intake of PO_4 is low, the active transport mechanism is dominant, and when the intake of PO_4 is high, the passive transport mechanism is dominant.

Calcium

Calcium is essential for optimal growth and bone development (NRC, 1998). Approximately 99% of the Ca in the body is located in the skeleton. Calcium in the skeleton is found in the structural complex hydroxyapatite along with P (Bronner, 1986; Ziegler and Filer, 1996; NRC 1998). Hydroxyapatite serves as a Ca reservoir for the rest of the body. Calcium homeostasis in the blood is regulated primarily by PTH, $1,25(OH)_2D_3$, and calcitonin, all of which will be discussed later. In addition, prostaglandins, reproductive steroids, and other hormones can affect Ca metabolism.

Calcium Absorption. Calcium absorption occurs throughout the small intestine *via* an active transcellular process or a paracellular process (Bronner et al., 1986). In order for Ca to be absorbed it must be solubilized within the intestinal lumen. The Ca in a given feedstuff may already be in solution or it may be solubilized through the actions of gastric enzymes and

peristalsis. The degree to which dietary Ca can be solubilized in the intestinal lumen is affected by many constituents within the diet. The formation of insoluble Ca-salts, such as $CaCO_3$ or Ca_3PO_4, is probably the most detrimental factor affecting Ca absorption. These salts, if present in the diet, dissociate in the stomach as a result of the low pH. However, as the digesta is passed caudally, through the small intestine, the pH increases, causing the salts to reform and precipitate out of solution. At neutral or higher pH very little Ca remains in solution (Washburn, 1928). The availability of Ca from limestone is markedly reduced if other Ca salts are present which are more soluble than $CaCO_3$. In other words, phosphate is more soluble than $CaCO_3$, therefore Ca from $CaCO_3$ will be substantially less available in the presence of phosphate, because less of the $CaCO_3$ will be in solution.

Transcellular Ca transport (Figure 1) occurs primarily in the proximal end of the small intestine (Bronner et al., 1986; Roche et al., 1986). It is an active process which occurs in three steps: uptake of Ca by the brush border membrane, transport of Ca through the cell, and movement of Ca out of the cell and into the blood stream. Evidence suggests that Ca enters the cell through a Ca channel (Homaidan et al., 1965; Hess and Tsien, 1984; Bronner et al., 1986; Caffrey and Farach-Carson, 1987; Butler and Hillier, 1989; Guggino et al., 1989; Saunders and Isaacson, 1990), traveling down an electrochemical gradient. Once in the cell, it binds to calbindin and diffuses through the cell to the basolateral membrane (Wasserman et al., 1968; Wasserman and Feher, 1977; Wasserman et al., 1978; Thomasett et al., 1982; Feher et al., 1992; Stein, 1992). Facilitated diffusion of Ca attached to calbindin is approximately 70 times faster than if free Ca were to diffuse through the cell as described by Fick's law (Stein, 1992). Movement of Ca out of the cell occurs up an electrochemical gradient, requiring energy in the form of ATP to drive the process. Two Ca transporters have been identified. The first is a Ca-

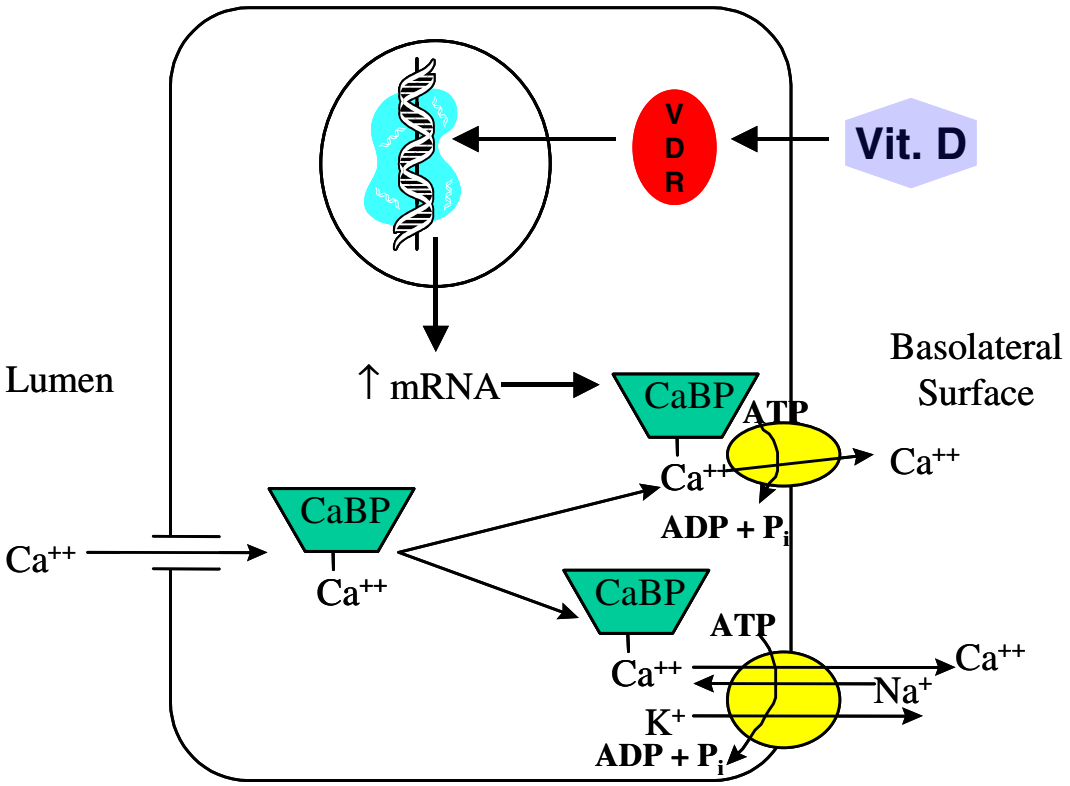


Figure 1. Transcellular Ca transport. CaBP = calcium binding protein and VDR = Vitamin D receptor (adapted from Weiser, 1984).

ATPase, which utilizes the energy derived from the hydrolysis of ATP to move Ca up the electrochemical gradient and out of the cell (Garrahan and Rega, 1990). Cytoplasmic binding sites for Ca and calmodulin have been identified (Verma et al., 1988; Carafoli et al., 1990). This transport mechanism is thought to be the primary transport mechanism for extrusion of Ca. The second transporter, found in many cells, is a Ca/Na antiport system (Reeves, 1990). Sodium is transported into the cell via a Na/K-ATPase and the inward movement of Na provides the energy necessary for Ca to be moved out of the cell. This process, however is not thought to play a major roll in Ca extrusion from duodenal cells (Nellans and Popovitch, 1984).

Paracellular transport (Figure 2) is a nonsaturable process which occurs throughout the small intestine (Ziegler and Filer, 1996). Ca uptake *via* paracellular transport is enhanced when hyperosmolar substances relative to body fluids are present in the diet. This causes an increased distention of the gut wall leading to an increased paracellular transport.

Therefore, Ca transport is mediated through two processes, both a saturable transcellular mechanism and a nonsaturable paracellular mechanism. This was described by Wasserman and Taylor (1969) using the following equation:

$$V_a = (V_m \times [Ca]_L) \div (K_m + [Ca]_L) + b[Ca]_L$$

where

V_a = amount of Ca absorbed per unit time
$V_m = V_{max}$ for the saturable component
$[Ca]_L$ = Ca concentration of the luminal fluid
$K_m = ½ V_{max}$
b = an apparent permeability constant

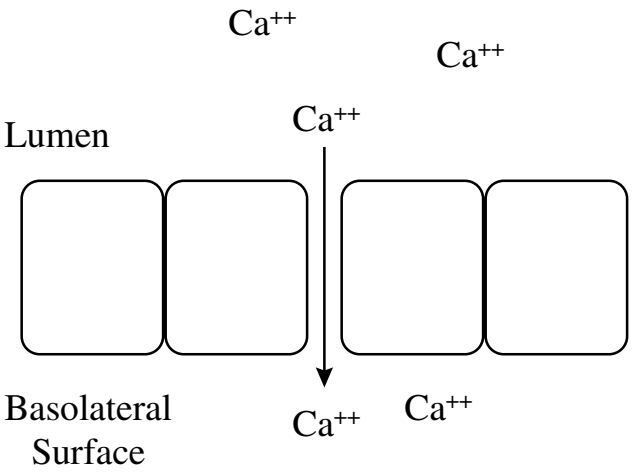


Figure 2. Paracellular Ca transport.

Regulation of Ca and P Metabolism

Calcium and P metabolism are primarily affected by serum Ca and P concentrations and three hormones: 1,25 dihydroxycholecalciferol ($1,25(OH)_2$-D3), parathyroid hormone (PTH), and calcitonin. Directly, 1,25 dihydroxycholecalciferol affects PO_4 metabolism by stimulating absorption *via* active transport mechanisms (Korwaski and Schachter, 1969; Chen et al, 1974; Kabakoff et al., 1982) and by stimulating bone resorption of both PO_4 and Ca (Korwaski and Schachter, 1969; Williams et al., 1989). Indirectly, $1,25(OH)_2$-D3, causes an increase in bone Ca resorption which leads to higher serum Ca levels, which decreases PTH levels, thus causing an increased reabsorption of PO_4 by the kidney (Amiel et al., 1970; Dominguez et al., 1976).

Low concentrations of P in the serum have been shown to stimulate reabsorption of P by the tubules of the kidney (Jongbloed, 1987; Ziegler and Filer, 1996; Berner, 1997). In addition, low serum P concentrations desensitize the kidney tubules to the effects of PTH. In contrast, decreased P reabsorption occurs when serum plasma levels are high. Steroid hormones have also been shown to decrease P reabsorption.

Parathyroid Hormone. Parathyroid hormone is released from the parathyroid gland in response to low blood Ca. It exerts its primary effects on bone and kidney. In both tissues it interacts with a plasma membrane receptor stimulating adenylate cyclase activity which produces the second messenger, cAMP (Aurbach, 1988; Habener and Potts, 1990). In bone, this causes a decrease in osteoblastic activity and an increase in osteoclastic activity (Bronner, 1996). This has a net result of increasing bone mineral resorption. In the kidney, cAMP concentrations in the urine are increased (Chase and Aurbach, 1967), urinary P excretion is increased, and Ca reabsorption from the distal convoluted tubule is increased (Massry, 1982). Indirectly, PTH

increases the intestinal absorption of Ca by stimulating the hydroxylation of 25-OH-D3 to $1,25(OH)_2$-D3 in the kidney (Norman and Litwack, 1987).

Calcitonin. Calcitonin is released from the parafollicular cells of the thyroid gland in response to high blood Ca. It interacts with a plasma membrane receptor found on osteoclasts (Chambers et al., 1985; Boyde and James, 1987) resulting in decreased osteoclastic activity (Malgaroli et al., 1989; Teti and Zallone, 1992) and thus a decreased bone resorption.

Vitamin D. The active form of vitamin D, 1α,25-dihydroxyvitamin-D_3 ($1,25(OH)_2$-D_3), is created through the dihydroxylation of vitamin D_3 (cholecalciferol). Vitamin D_3 can be endogenously produced in the skin through the photoconversion of 7-dehydrocholesterol, or it can be obtained from the diet as cholecalciferol or synthetic vitamin D. The two hydroxylation steps that transform vitamin D to the active form of vitamin D occur in the liver and the kidney. In the liver, 25-hydroxylase catalyzes the addition of a hydroxyl group to carbon 25. In the kidney, 25-hydroxyvitamin-D_3-1α-hydroxylase catalyzes the addition of a hydroxyl group to carbon 1, creating the active form of vitamin D, $1,25(OH)_2$-D_3. Alternatively, 25(OH)-D_3 can be hydroxylated at carbon 24 to create $24,25(OH)_2$-D_3 which does not possess the metabolic activity of $1,25(OH)_2$-D_3, and can ultimately be further metabolized and excreted in bile. The primary function attributed to $1,25(OH)_2$-D_3 is its role in Ca homeostasis. While the function of vitamin D in Ca homeostasis has been known for many years, the mechanisms through which these actions occur have only recently been established.

Vitamin D can transcriptionally regulate genes containing a vitamin D response element (VDRE). This VDRE consists of two nucleotide sequence repeats of AGGTCA (A = adenine, T = thymine, G = guanine, C = cytosine) separated by three bases (Umesono et al., 1991). In order for vitamin D to act in *trans* upon this *cis*-acting response element it must bind to a vitamin D

receptor (VDR) protein. The VDR is an approximately 50 kDa protein which can be divided into four functional domains: 1) a N-terminal domain of unknown function, 2) a DNA binding domain, 3) a hinge region, and 4) a C-terminal ligand binding domain (McDonnell et al., 1987; Baker et al., 1988; Burmester et al., 1988; Elaroussi et al., 1994; Kamei et al., 1995). The ligand binding domain is the largest region of the protein, consisting of approximately 70% of the total amino acids. This domain is responsible for binding $1,25(OH)_2$-D_3, transcription factors, and for homeric or hetreomeric dimerization. The effects of $1,25(OH)_2$-D_3 mediated through VDR requires heterodimerization of VDR with the 9-*cis* retinoic acid receptor, RXR (Kliewer et al., 1992; Forman et al., 1995) The presence of $1,25(OH)_2$-D_3 increases the heterodimerization of VDR and RXR (Kimmel-Jehan et al., 1997); while the presence of 9-*cis* retinoic acid may decrease the heterodimerization of VDR and RXR and increase the homodimerization of RXR (Jones et al., 1998). In addition to requiring the VDR-RXR heterodimerization to potentiate the transcriptional actions of $1,25(OH)_2$-D_3, multiple coactivator proteins are needed. Coactivator proteins include transcription factor IIB (TFIIB), whose binding overlaps the c-terminal side of the hinge region and the N-terminal end of the ligand binding region (Blanco et al., 1995; MacDonald et al., 1995). The actions of $1,25(OH)_2$-D_3 are mediated through binding to a VDR-RXR heterodimer. This $1,25(OH)_2$-D_3-VDR-RXR complex binds to the VDRE of vitamin D responsive genes, and with the interaction of various coactivators and transcription factors, initiates transcription of the gene.

Secretion of PTH increases in response to low serum Ca levels. The PTH acts upon receptors in the proximal convoluted tubules of the kidney to increase 25-hydroxyvitamin D_3-1α-hydroxlase mRNA through a cAMP dependent manor (Garabedian et al., 1972; Tanaka et al., 1975; Norman and Litwack, 1987; Shinki et al., 1997). In addition, PTH causes a decreased

synthesis of 25-hydroxyvitamin D_3-24-hydroxylase (Shinki et al., 1992). The net result is an increased output of the active form of vitamin D, 1,25$(OH)_2$-D_3, which acts to increase serum Ca through direct effects on the intestine, kidney, and bone.

In the intestine, 1,25$(OH)_2$-D_3, causes an increased absorption of Ca. The effects of vitamin D on Ca uptake are due to its effects on Ca transport through the cell, and transport across the basolateral membrane. Calcium transport through the cell is aided by the Ca binding protein, calbindin D-9k in mammals (Umesono et al., 1991), and calbindin D-28k in birds (Christakos, et al., 1997). Both transport proteins have been shown to increase in response to 1,25$(OH)_2$-D_3 (Umesono et al., 1991). The gene encoding calbindin D-9k has been cloned from rat intestine and contains a VDRE, which suggests that 1,25$(OH)_2$-D_3 acts through transcriptional regulation to increase calbindin (Thomasett, 1997). However, no VDRE has been identified in the avian calbindin d-28k gene. Therefore, the mechanisms through which 1,25$(OH)_2$-D_3 stimulates an increase in calbindin are still unclear. Two transporters have been identified which are responsible for moving Ca across the basolateral membrane against an electrochemnical gradient. The first is a Ca-ATPase, which is thought to be the primary mechanism for Ca extrusion across the basolateral membrane (Garrahan and Rega, 1990). The second, which is thought to only have a minor role in Ca transport across the basolateral membrane, is a Ca/Na antiport system (Reeves, 1990). Wasserman et al. (1992) provided evidence which suggests that the Ca-ATPase may be inducible by 1,25$(OH)_2$-D_3.

In the kidney, 1,25$(OH)_2$-D_3 has two major functions. First, it increases Ca reabsorption in the distal convoluted tubule, and second, it down regulates 1,25$(OH)_2$-D_3 production. The actions of 1,25$(OH)_2$-D_3 on Ca reabsorption, much like in the intestine, appear to be mediated primarily through an increased transcription of the gene encoding the renal Ca transport protein,

calbindin D-28k. This mammalian D-28k calbindin gene contains a VDRE (Gill and Christakos, 1993). Down regulation of $1,25(OH)_2$-D_3 production is a two-pronged effect which includes the down regulation of 25-hydroxyvitamin-D_3-1α-hydroxylase and the up regulation of 25-hydroxyvitamin-D_3-24-hydroxylase. The down regulation of 25-hydroxyvitamin-D_3-1α-hydroxylase may be through a direct action of $1,25(OH)_2$-D_3 complexed with VDR and RXR on a VDRE, or it may be indirectly through a down regulation of PTH production due to the direct actions of $1,25(OH)_2$-D_3 on the parathyroid gland. Activity of 25-hydroxyvitamin-D_3-24-hydroxylase is upregulated in the proximal and distal convoluted tubules by $1,25(OH)_2$-D_3 (Yang et al., 1999). However, the effects of $1,25(OH)_2$-D_3 are confounded by levels of PTH and/or cAMP which display differential effects in the proximal and distal convoluted tubules (Yang et al., 1999).

The principle function of $1,25(OH)_2$-D_3 in bone is to increase Ca resorption by increasing osteoclastic activity, increasing the movement of Ca from the bone into the bone fluid compartment, and increasing the movement of Ca from the bone fluid compartment into the plasma. All of these mechanisms of action require the presence of PTH. Osteoclastic bone resorption increases in response to $1,25(OH)_2$-D_3 and PTH (Raisz et al., 1972; Stern, 1997). However, osteoclasts do not possess receptors for $1,25(OH)_2$-D_3 or PTH (Jones et al., 1998). Instead, $1,25(OH)_2$-D_3 and PTH act through receptors on osteoblasts. They cause a "rounding up" of osteoblasts through a cascade of cytoskeletal changes, which have yet to be elucidated (Suda and Takahashi, 1997). This results in the osteoblasts covering a smaller surface area of the bone which allows the osteoclasts to spread out, covering more surface area, and resorbing more bone. In addition, $1,25(OH)_2$-D_3 causes osteoblastic production of an osteoclastic differentiation

factor that causes the osteoclastic precursor, the monocyte, to differentiate into a mature osteoclast (Abe et al., 1981; Tanaka et al., 1982; Suda and Takahashi, 1997).

Finally, $1,25(OH)_2$-D_3 may act directly on the parathyroid gland. The parathyroid gland has been shown to contain VDR (Hughes and Haussler, 1978), and the PTH gene has recently been shown to contain VDRE (Demay et al., 1992). Therefore, $1,25(OH)_2$-D_3 may regulate production of PTH and the actions of PTH in the kidney and in bone by decreasing the transcription of the PTH gene through a $1,25(OH)_2$-D_3-VDR-RXR interaction with the VDRE in the promoter region of the PTH gene.

In summary, the primary function of $1,25(OH)_2$-D_3 is in the regulation of Ca homeostasis. Target organs for $1,25(OH)_2$-D_3 relevant to Ca homeostasis, include the small intestine, kidney, bone, and parathyroid gland. The mechanisms through which $1,25(OH)_2$-D_3 works are complex and only partially understood. The $1,25(OH)_2$-D_3, through an interaction with a VDR-RXR heterodimer, can stimulate or inhibit gene transcription with the aid of various coactivators and transcription factors, which are only now beginning to be elucidated.

Response Criteria used to Measure Ca and P Status

Criteria which are often used to measure the response of pigs to varying levels of P or Ca include performance measurements (average daily gain, feed intake and feed efficiency), blood measurements (serum P and Ca concentrations), bone criteria (ash percentage, shear force, bending moment) and digestibility estimates. Pigs have a higher Ca and P requirement for bone mineralization than they do for growth (NRC, 1998). Therefore, responses of bone criteria can be seen over a wider range of dietary Ca and P than responses to growth.

Several studies have found that growth rate, feed intake, and feed efficiency are not sensitive indicators of Ca and P status (Doige et al., 1975; Crenshaw, 1981; Kornegay, 1981; Koch and Mahan, 1985). However, when Ca and P are fed at low levels, responses in growth rate and feed efficiency have been seen (Miller et al., 1962, 1964; Cromwell et al., 1970; Doige et al., 1975; van Kempen et al., 1976; Ross et al., 1984). In studies of Kornegay and Qian (1996) and Yi et al. (1996c) in which the effects of added phytase and P to a low P basal diet were investigated, growth rate and feed intake were moderately sensitive indicators of the effects of both dietary phytase and P.

Serum Ca and P concentrations, similar to performance criteria, are primarily responsive when low levels of Ca and/or P are fed (Doige et al., 1975; Reinhard et al., 1976; Kornegay and Thomas, 1981; Koch and Mahan, 1985). Serum P has been shown to increase while serum Ca decreases as the level of P in the diet is increased (Miller et al., 1964; Cromwell et al., 1970; Kornegay and Thomas, 1981). The adverse effects of a wide Ca:P ratio can be seen in serum. As the Ca:P ratio widens, serum P concentration decrease and serum Ca concentration increases (Koch et al., 1984; Koch and Mahan, 1985).

Kornegay (1985) and Peo (1991) both concluded that bone parameters were more responsive to Ca and P over a wider range of dietary Ca and P than performance or blood parameters. Mechanically, effects on bones are most often measured by shear force and bone bending moment due to the simplicity of these tests (Kornegay, 1985; Peo, 1991). In several studies it was found that bone bending moment is a very sensitive test for Ca and P status (Dellaert et al., 1990; Keteran et al., 1993a,b; Cromwell et al, 1993, 1995). Combs et al. (1991a,b) however, found that bone shear force was even more sensitive than bone bending moment. Bone ash is also a very simple, but reliable test of bone mineralization. Rutlege et al.

(1961) and Miller et al. (1962, 1964) found a high correlation between bone ash and dietary P levels in baby pigs. In several studies the same correlation was demonstarted in weanling pigs (Combs et al., 1962; Hoppe et al., 1992) and grower-finisher pigs (Cromwell et al., 1970; Pond et al., 1975; Mahan et al., 1980).

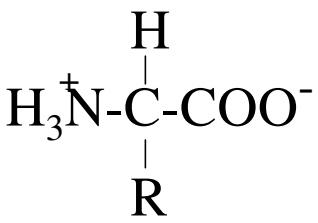
Typically, metacarpals, metatarsals and femurs have been the bones used to assess Ca and P status. However, in studies by Kornegay and Qian (1996) and Yi et al. (1996c) the 10^{th} rib was more highly correlated with the response equations to P and phytase than the metacarpal.

The use of apparent P digestibility as an estimate of P bioavailability in feedstuffs has been used frequently in recent studies where microbial phytase was added to the diet (Pointillart et al., 1984, 1987; Simons, 1990; Eeckhout and de Paepe, 1991; Jongbloed et al., 1992; Dungelhoef et al., 1994; Pallauf et al, 1992, 1994; Mroz et al., 1994; Kornegay and Qian, 1996; Yi et al., 1996c, Radcliffe and Kornegay, 1998; Rice et al., 1999; Skaggs et al., 1999; Zhang and Kornegay, 1999; Rice et al., 2000; Robbins et al., 2000). This is supported by the conclusions of Dellaert et al., (1990) who compared eight P supplements added to supply 0.6 to 2.2 g/kg of additional P to a basal diet which contained 3.0 to 3.2 g/kg of P. In addition, a comparison of techniques used to evaluate P availability from feedstuffs was made. They concluded that apparent P digestibility was the most sensitive indicator of P bioavailability, followed by bone parameters, with blood parameters showing an insufficient response to P. However, Peo (1991) concluded that apparent Ca and P digestibilities were of no use in estimating their bioavailability in feedstuffs. In the studies of Kornegay and Qian (1996) and Yi et al. (1996c) where the effects of phytase were investigated, the apparent digestibility of P did serve as a good measure of the bioavailability of P from the feedstuff. This was further supported by the work of Radcliffe and Kornegay (1998), Skaggs (1999), and Zhang (1999).

Ross et al. (1984) reported data from five studies investigating the bioavailability of Ca from various Ca supplements in pigs with an average starting weight of 15 kg. They concluded that bone breaking strength was the most sensitive indicator of Ca status as it provided a linear response to Ca over the widest range (.05 to .65% total Ca). However, they found that mortality was high at the lowest level of Ca and that the curve appeared to plateau at the highest level of Ca. They concluded that the most desirable response surface for Ca occurred between .20 and .50% total Ca. In the other four experiments presented by Ross et al. (1984), they found that bone strength and ash always increased linearly as the Ca level in the diet increased form .20% to .35% to .50%. Performance data gave variable responses, with some experiments showing improvements in weight gain and feed efficiency and others showing no effect as the Ca level in the diet was increased. They found no differences in the bioavailability of Ca from calcitic limestone, oyster shell flour, gypsum, marble dust, and aragonite. Calcium from dolomitic limestone was less available than Ca from any of the other sources.

Protein

Amino acids serve as the building blocks for proteins. Approximately 20 amino acids are found naturally in feed ingredients fed to pigs (NRC, 1998). Each amino acid consists of a central carbon atom bonded to an amino group, a carboxyl group, a hydrogen atom, and an "R" group. The composition of the R group designates the particular amino acid. The general structure of all amino acids is as follows:

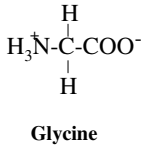


Amino acids can be subdivided into five classes based on the composition of the R groups. They include amino acids with nonpolar, aliphatic R groups, polar, uncharged R groups, aromatic R groups, positively charged R groups, and negatively charged R groups (Table 1).

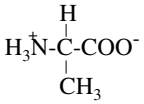
Interest has continued to grow in maximizing feed efficiency and concerns over the environment have become an important issue in production agriculture. Therefore, interest has grown in diet formulation to meet amino acid requirements rather than formulating diets to meet the crude protein requirement. Formulating a diet on crude protein means that enough protein needs to be added to the diet to meet the requirement for the most limiting amino acid in the diet. As a result, diets formulated on crude protein are over formulated for amino acids except the most limiting amino acid. One of the concepts that has grown out of this initiative to formulate diets based on amino acid requirements rather than crude protein requirements has been the "ideal protein" concept. The basic premise behind the ideal protein concept is that the "perfect" or "ideal" protein would have a ratio of amino acids that identically matches the levels needed by the pig. To develop this ratio of amino acids, it is necessary to set one amino acid at 100% and calculate the relative proportions of other amino acids to that one standard amino acid. Most researchers have used lysine because it is the most limiting amino acid in typical swine diets based on corn and soybean meal (NRC, 1998). One problem with the ideal protein concept is

Table 1. Amino acid classification and structure.

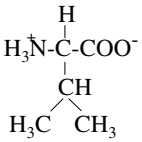
Nonpolar, aliphatic R groups



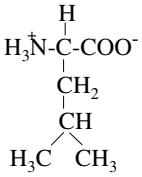
Glycine



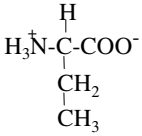
Alanine



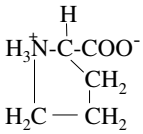
Valine



Leucine

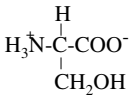


Isoleucine

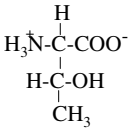


Proline

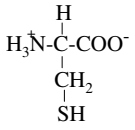
Polar, uncharged R groups



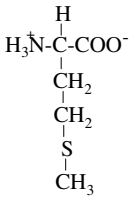
Serine



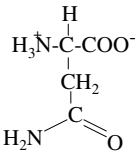
Threonine



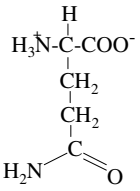
Cysteine



Methionine

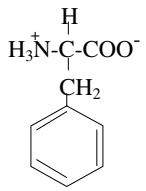


Asparagine

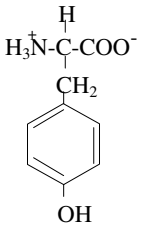


Glutamine

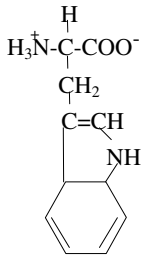
Aromatic R groups



Phenylalanine

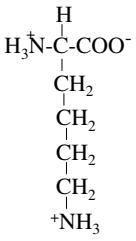


Tyrosine

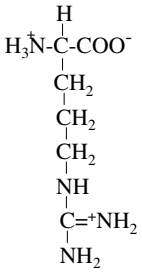


Tryptophan

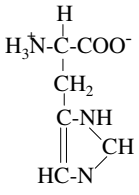
Positively charged R groups



Lysine

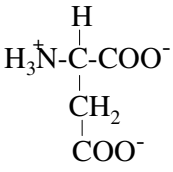


Arginine

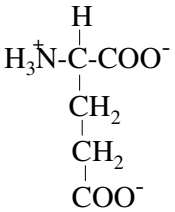


Histidine

Negatively charged R groups



Aspartate



Glutamate

that it does not exist in nature, and it is too expensive to manufacture. However, methods of manufacturing crystalline amino acids have become more cost effetive in recent years, and it is now economically feasible for producers to add several crystalline amino acids to the diet. As a result, the dietary crude protein level can be decreased.

To more closely balance the diet to meet amino acid requirements, there is growing interest in formulating diets to meet amino acid requirements based on available levels of amino acids in the diet as is commonly done with P. This requires correct estimates of the requirements of each amino acid, and a proper estimation of the bioavailability of each amino acid from each feedstuff used in the diet.

Jonderville et al. (1995) reviewed the findings of a multi-year study commissioned by Eurolysine (16 rue Balle, 75424 Paris, France) and the Technical Institute for Cereals and Forages (8 avenue du Président Wilson, 75116 Paris, France) that evaluated the amino acid bioavailability of 142 batches of feedstuffs using ileo-rectal anastomosed pigs. They determined the apparent ileal digestibility of each test ingredient and then adjusted the apparent digestibility value to a standardized or true digestibility value by adjusting for non-specific and diet specific endogenous amino acid losses.

The NRC (1998) reviewed data from Heartland Lysine (Heartland Lysine, 1995), BioKyowa (Southern, 1991), Rhône-Poulenc (Rhône-Poulanc, 1993), and Eurolysine (Jondreville, 1995), to estimate amino acid bioavailability. Their estimates for amino acid apparent ileal digestibility and true ileal amino acid digestibility are listed in Tables 2 and 3. In addition, the estimates of true and apparent ileal amino acid digestibilities determined by Jondreville et al. (1995) are shown. At the bottom of each table the difference between the two

Table 2. Estimates of apparent ileal digestibility of amino acids from various feedstuffs.

	Apparent ileal amino acid digestibilities, %											
Item	Arg	His	Ile	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Trp	Val
NRC, 1998												
Corn	83	82	79	88	66	86	78	83	83	69	64	79
SBM, 44% CP	91	86	84	84	85	86	77	85	86	78	80	81
SBM 48% CP	90	86	84	84	85	86	79	84	85	78	81	81
Wheat, soft red	83	84	84	85	73	85	84	87	84	72	81	80
Wheat midds	88	76	77	78	75	82	82	83	83	69	77	76
Canola meal	81	80	74	78	74	82	79	76	73	69	73	71
Sorghum	78	73	80	86	62	81	79	81	83	68	75	78
Meat and bone meal	81	75	74	76	74	79	55	76	71	70	60	74
Jondreville et al., 1995												
Corn	86.2	84.5	82.5	89.6	72.2	88.5	83.3	87.2	85	75	64.4	80.8
SBM, 44% CP												
SBM 48% CP	93.5	89.7	88.4	87.8	88.5	89.8	83.5	89.2	89.8	83.5	85.9	85.7
Wheat	85.1	87.1	85.4	86.1	76.5	86.4	87.5	88.7	86.5	77.2	83.1	80.9
Wheat midds	92.7	93.3	89.3	91	85	91.5	90.2	92.3	89.1	84.5	86.3	87.2
Canola meal	77.1	80	72.6	75.6	72.1	83.5	76.2	76.4	73.3	70.2	70.9	70.8
Sorghum	76.7	73.4	77	83.6	63	81.4	71.2	80.6	79.7	67.6	71.9	74.2
Meat and bone meal	73.5	81.6	74.6	76.7	74.9	78.7	49.8	72.5	64.2	72.3	66.9	72
Difference = (Jondreville et al.values) - (NRC values)												
Corn	3.2	2.5	3.5	1.6	6.2	2.5	5.3	4.2	2	6	0.4	1.8
SBM, 44% CP												
SBM 48% CP	3.5	3.7	4.4	3.8	3.5	3.8	4.5	5.2	4.8	5.5	4.9	4.7
Wheat, soft red	2.1	3.1	1.4	1.1	3.5	1.4	3.5	1.7	2.5	5.2	2.1	0.9
Wheat midds	4.7	17.3	12.3	13.0	10.0	9.5	8.2	9.3	6.1	15.5	9.3	11.2
Canola meal	-3.9	0.0	-1.4	-2.4	-1.9	1.5	-2.8	0.4	0.3	1.2	-2.1	-0.2
Sorghum	-1.3	0.4	-3	-2.4	1	0.4	-7.8	-0.4	-3.3	-0.4	-3.1	-3.8
Meat and bone meal	-7.5	6.6	0.6	0.7	0.9	-0.3	-5.2	-3.5	-6.8	2.3	6.9	-2.0

Table 3. Estimates of true ileal digestibility of amino acids from various feedstuffs.

	True ileal amino acid digestibilities, %											
Item	Arg	His	Ile	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Trp	Val
NRC, 1998												
Corn	89	87	87	92	78	90	86	90	89	82	84	97
SBM, 44%	93	90	88	88	89	91	84	88	90	85	87	86
SBM 48%	94	91	89	89	90	91	87	89	90	87	90	88
Wheat, soft red	88	89	89	89	81	90	90	91	89	84	90	86
Wheat midds	95	94	92	93	89	93	91	95	92	88	91	90
Canola meal	85	85	78	81	78	86	83	82	79	76	75	77
Sorghum	87	81	87	90	81	89	83	88	87	84	83	87
Meat and bone meal	83	83	82	81	80	83	63	81	78	80	78	79
Jondreville et al., 1995												
Corn	90.8	88.1	88.1	92.2	81.1	92.6	88.4	92	88.8	82.1	76	86.1
SBM, 44%												
SBM 48%	94.9	91.5	90.7	90	90.9	93.3	87.5	91.8	92	87.1	89.9	88.6
Wheat	88.1	89.7	89.7	89.8	83.3	90.6	91.3	92.7	90.1	83.9	88.5	85.5
Wheat midds	95.5	95.8	93.3	94.3	90.9	95	93.9	96	92.6	90.4	91.5	91.1
Canola meal	79	81.7	75.2	78	74.7	85.7	78.8	79.6	76	73.3	74.6	73.5
Sorghum	82.7	77.7	82.2	85.9	74.6	86.2	77.6	85.4	83.4	75.4	79.9	79.2
Meat and bone meal	75.1	84.1	79.2	80.1	75	80.4	54.2	77.5	69	75.2	77.6	76
Difference = (Jondreville et al.values) - (NRC values)												
Corn	1.8	1.1	1.1	0.2	3.1	2.6	2.4	2	-0.2	0.1	-8	-10.9
SBM, 44%												
SBM 48%	0.9	0.5	1.7	1.0	0.9	2.3	0.5	2.8	2.0	0.1	-0.1	0.6
Wheat, soft red	0.1	0.7	0.7	0.8	2.3	0.6	1.3	1.7	1.1	-0.1	-1.5	-0.5
Wheat midds	0.5	1.8	1.3	1.3	1.9	2.0	2.9	1.0	0.6	2.4	0.5	1.1
Canola meal	-6.0	-3.3	-2.8	-3.0	-3.3	-0.3	-4.2	-2.4	-3.0	-2.7	-0.4	-3.5
Sorghum	-4.3	-3.3	-4.8	-4.1	-6.4	-2.8	-5.4	-2.6	-3.6	-8.6	-3.1	-7.8
Meat and bone meal	-7.9	1.1	-2.8	-0.9	-5.0	-2.6	-8.8	-3.5	-9.0	-4.8	-0.4	-3.0

predictions as calculated by the values of Jondreville et al. (1995) minus the values estimated by the NRC (1998) are shown.

Differences between the two sources estimating amino acid bioavailability ranged from -7.8 to 17.3 percentage units for apparent ileal digestibility and from -8.8 to 2.9 percentage units for true ileal digestibility (Jondreville et al., 1995; NRC, 1998). These differences are large considering that data from Jondreville et al. (1995) was included in the data set used by the NRC (1998) to estimate apparent and true ileal amino acid digestibility. The average apparent amino acid digestibility estimates differed by $\pm$3.3, $\pm$4.4, $\pm$2.4, $\pm$10.5, $\pm$1.5, $\pm$2.3, and $\pm$3.6 for corn, soybean meal (48%), wheat, wheat middlings, canola meal, sorghum, and meat and bone meal, respectively. True amino acid digestibility estimates differed by an average of $\pm$2.8, $\pm$1.1, $\pm$0.95, $\pm$1.4, $\pm$2.9, $\pm$4.7, and $\pm$4.2 for corn, soybean meal (48%), wheat, wheat middlings, canola meal, sorghum, and meat and bone meal, respectively. Therefore, the variability of amino acid bioavailability estimates decreased for corn, soybean meal (48%), wheat, and wheat middlings, but increased for canola meal, sorghum, and meat and bone meal when apparent ileal digestibilities were converted to true ileal digestibilities.

The method of ileal digesta collection undoubtedly has the potential to affect estimates of amino acid digestibility. It is essential that an accurate, repeatable method be used to collect digesta that does not interfere with the normal digestive processes of the pig. Methods for ileal digesta collection will be discussed in subsequent sections along with the advantages and disadvantages of each method

Amino Acid Absorption. Historically, it was assumed that before absorption across the brush border membrane, ingested proteins were completely broken down into free amino acids within the lumen of the gastrointestinal tract. Free amino acids are transported across the brush

border membrane by a number of amino acid transporters many of which require the co-transport of Na^+ to make the transport energetically favorable. However, in 1976, Mathews and Adibi hypothesized that at least one peptide transporter must exist. This hypothesis was based on the results of two earlier experiments (Adibi, 1971; Adibi and Soleimanpour, 1974) where human volunteers were infused with unhydrolyzable peptides in the upper small intestine and recovered in the lower small intestine. They found that a significant amount of these di- and tri-peptides were disappearing in the small intestine. In addition, Adibi and Soleimanpour (1974) also discovered that that addition of more di- and tri-peptides inhibited the absorption of some of the di- and tri-peptides within their infusate. However, absorption was not affected by the addition of free amino acids or by peptides in excess of three amino acids in length. These results led them to conclude that there was an intestinal, brush border membrane, peptide transporter in the small intestine.

Their hypothesis was met with skepticism, but the observation that patients with defects in their brush border membrane amino acid transport proteins had no clinical signs of protein deficiency led researchers to further investigate the possibility of a peptide transporter. In 1994, Fei et al. were the first group to clone a rabbit peptide transporter called PepT1 from intestinal cDNA. Prior to this, many research groups investigated the kinectics of peptide transport using *in vitro* systems.

Characterization of Peptide Transport Using In Vitro Systems. Rajendran et al. (1985, 1987), using human brush border membrane vesicles (BBMV), found that the dipeptide, Gly-Pro, and the tripeptide, Gly-Gly-Pro, were transported across the membrane into the cytoplasm. In addition, adding di- and tri-peptides to the medium inhibited the transport of these peptides across the membrane. The addition of free amino acids to the medium had no effect on transport

rate. Mackenzie et al. (1992), also using human BBMV, observed that the transport of the dipeptide, Gly-Gln, across the membrane was increased when an outward to inward H^+ gradient was imposed. The above research groups concluded that peptide transport was not *via* an active process because they were unable to produce an overshoot when imposing a Na^+ or H^+ gradient.

Several other researchers used Caco-2 cell lines to investigate peptide transport which were shown to be a suitable model for investigating peptide transport across the human intestinal brush border membrane (Hidalgo et al., 1989; Hidalgo and Borchardt, 1990). Thwaites et al. (1993) observed a marked acidification intracellularly with the inward transport of the dipeptide, Gly-Sar, across the membrane of Caco-2 cells. This suggested that peptide transport occurs via an active mechanism as initially proposed by Mathews and Adibi (1976).

Cloning of PepT1 cDNA. In 1994, Fei et al. reported the cloning of a rabbit peptide transporter called PepT1 from rabbit intestinal cDNA. The cDNA encoding rabbit PepT1 contained a 2121 bP open reading frame that encoded 707 amino acids. Based on the amino acid sequence, PepT1 is proposed to contain 12 transmembrane domains with a large hydrophilic, extracellular loop between domains nine and ten.

Liang et al. (1995), using a probe derived from the rabbit PepT1 cDNA sequence, screened a human intestinal cDNA library and isolated the cDNA sequence encoding human PepT1. The sequence showed a high degree of homology to rabbit PepT1, encoding 708 amino acids. In addition, the amino acid sequence predicted 12 transmembrane segments with a large, extracellular, hydrophillic loop between domains nine and ten. Seven putative N-linked glycosylation sites were identified within the extracellular loop. In addition, two possible sites for protein C-dependent glycosylation were observed.

Saito et al. (1995) and Miyamato et al. (1996) cloned and characterized rat PepT1 cDNA. They found high homology between rat, rabbit and human cDNA. The transmembrane domains in particular, were highly conserved.

Characterization of PepT1 Using Expression Vectors. The cloning of PepT1 cDNA allowed many researchers to investigate the kinectics of the peptide transporter through *in vitro* expression systems. Mackenzie et al. (1996) used Xenopus laevis oocytes expressing human PepT1 to characterize transporter kinectics. Using the voltage-clamp technique, they were able to show that for each peptide transported across the membrane, one H^+ was cotransported. This confirmed that the transport of peptides across the brush border membrane through the peptide transporter was energy dependent. Furthermore, they quantified the co-transport of one H^+ with each peptide, regardless of peptide size (di- or tri-peptide) or charge.

Cloning of Rat Genomic PepT1 DNA. Shiraga et al. (1999), using a partial sequence of rat PepT1 cDNA, was the first to isolate and clone rat genomic PepT1 DNA. They isolated and cloned the promoter region with a set of nested deletions into plasmid pGL3 containing the luciferase reporter gene. This, combined with promoter sequence information, led them to conclude that the rat PepT1 promoter contains a TATA box and a GC box 22 and 39 bp upstream from the transcription start site, respectively. Furthermore, they found possible response elements for AP-1, AP-2, octamer binding protein, and amino acids upstream from the transcription start site.

Understanding how PepT1 expression is regulated is important from a nutritional point of view as well as from a therapeutic standpoint. Much research is now focusing on the uptake of peptido-mimetic drugs as an alternative to intravenous administration. The recent characterization of the rat PepT1 promoter region by Shiraga et al. (1999) has provided insight

into possible sites of transcriptional regulation. This supports early findings of Erickson et al. (1995) who found a two-fold increase in rat intestinal PepT1 mRNA when rats were switched from a low crude protein diet to a high crude protein diet. In addition to transcriptional regulation, Thamotharan et al. (1996) suggested that PepT1 may also be regulated by post-translational mechanisms. They provided evidence suggesting that increased insulin concentrations cause an increase membrane insertion of PepT1 from a preformed cytoplasmic pool as occurs with the glucose transporter, Glut4.

Identification of PepT1 mRNA in Swine. In 1999, Chen et al., using primers which were designed based on the rabbit PepT1 cDNA sequence, isolated a 446 bp fragment of ovine PepT1 by RT-PCR amplification of ovine intestinal mRNA. They cloned and sequenced this fragment and used it to probe mRNA isolated from various tissues in poultry, dairy cows, and swine. In swine they isolated mRNA from the stomach, intestinal sections, liver, kidney, and semitendinosous and longissimus sections. Their probe hybridized only in the small intestine. Interestingly, their probe hybridized to two bands, 3.5 and 2.9 kb in size. In all other species only one band was observed. This suggests that the pig either has two closely related genes encoding peptide transporters or that some form of alternative RNA splicing may be occurring. Additionally, they found the highest concentration of PepT1 mRNA in the jejunum, followed by the duodenum, then the ileum.

As research into amino acid digestibility continues, it will be necessary to consider protein degradation, free amino acid absorption, and peptide absorption. The concept of the "ideal protein" has gained much interest in recent years. Potentially, a protein could be developed that has the ideal ratio of amino acids. This concept seems sound, but ultimately

protein utilization in the diet may be far more complex. It may be necessary to investigate peptide availability and requirements separately from amino acid availability and requirements.

Phytate

Phytic acid ($C_6H_{18}O_{24}P_6$), formally know as myoinositol 1, 2, 3, 4, 5, 6 hexa, dihydrogen phosphate (IUPAC-IUB, 1975) is shown in Figure 3. Phytate, the salt of phytic acid, serves as the primary storage form of P in plants, accounting for 60 to 80% of the total P. Reported values in the literature for the total P content and the phytate-P content of various plant ingredients are shown in Table 4.

Monogastric animals do not secret phytase in sufficient quantities to breakdown the phytate molecule, hence, the P incorporated in the phytate molecule is not available for absorption. Therefore, farmers must add large amounts of a highly available inorganic P source to meet the P requirement. The unavailable phytate P is excreted, which potentially can lead to environmental pollution problems. Estimates of the bioavailability of P from various plant ingredients as reported in the literature are shown in Table 5.

Mineral Chelating Ability of Phytic Acid. At a neutral pH phytic acid has been shown to carry one or two negatively charged oxygen atoms in each phosphate group (Erdman, 1979), giving it a total of up to 12 negative charges. Because of these negative charges, phytic acid has the ability to bind with a variety of di and tri-valent cations including Ca, Mg, Zn, Cu, Fe, Co, and Cr, in the small intestine of the pig and amino acids or peptides rendering them unavailable for absorption (Maga, 1982; Reddy et al., 1982; Morris 1986). The mineral complexing potential of phytic acid is shown in Figure 4.

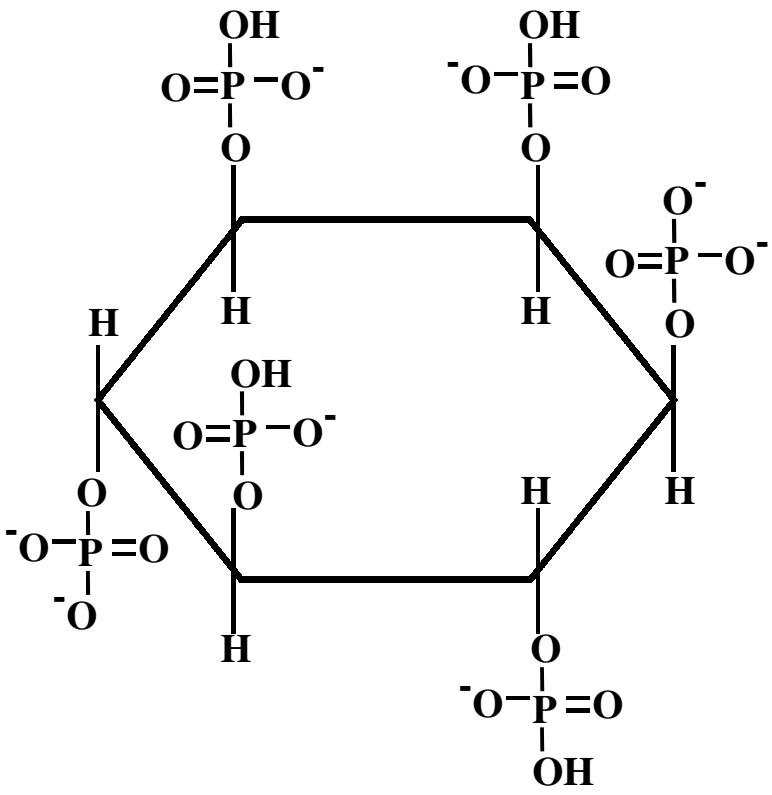


Figure 3. Structure of phytic acid.

Table 4. Total and phytate P content of various feedstuffs.

Feedstuff	Total P, %	Phytate P, % of total	Reference
Corn	.28		NRC, 1998
Corn	.26	66	Nelson et al., 1968
SBM, 44%	.65		NRC, 1998
SBM, 48.5%	.69		NRC, 1998
SBM	.61	61	Nelson et al., 1968
SBM, 44%	.66	53	Eeckhout and De Paepe, 1994
SBM, 48%	.61	52	Eeckhout and De Paepe, 1994
SBM		51-61	Pointillart, 1994
Barley	.35-.36		NRC, 1998
Barley	.34	56	Nelson et al., 1968
Barley	.37	60	Eeckhout and De Paepe, 1994
Barley		51-66	Pointillart, 1994
Wheat	.35-.39		NRC, 1998
Wheat	.30	67	Nelson et al., 1968
Wheat	.33	67	Eeckhout and De Paepe, 1994
Wheat		60-77	Pointillart, 1994
Wheat middlings	.93		NRC, 1998
Wheat middlings	.80	66	Eeckhout and De Paepe, 1994
Canola meal	1.01		NRC, 1998
Canola meal	1.12	36	Eeckhout and De Paepe, 1994
Sorghum	.29		NRC, 1998
Sorghum	.27	70	Eeckhout and De Paepe, 1994
Sorghum	.31	68	Nelson et al., 1968
Meat and bone meal	4.98		NRC, 1998

Table 5. Estimates of P bioavailability from various feedstuffs for pigs.

Source	Bioavailability[a], %	Standard[b]	Reference
Corn	14	MSP	NRC, 1998
Corn	14	MSP	Cromwell, 1992
Corn	15		Pierce et al., 1977
Corn	12		Calvert et al., 1978
Corn	29		Pointillart, 1984
Corn	48		Pointillart, 1987
Corn	29	MSP	Huang and Alle, 1981
SBM, 44%	31	MSP	NRC, 1998
SBM, 48%	23		
SBM, 44%	31	MSP	Cromwell, 1992
SBM, 44%	27		Tonroy et al., 1973
SBM, 44%	36	MSP	Huang and Alle, 1981
Barley	30	MSP	NRC, 1998
Barley	30	MSP	Cromwell, 1992
Barley	28		Calvert et al., 1978
Wheat	50	MSP	NRC, 1998
Wheat	49	MSP	Cromwell, 1992
Wheat	46		Pointillart, 1984
Wheat	51	MSP	Huang and Allee, 1981
Wheat middlings	41	MSP	NRC, 1998
Wheat middlings	41	MSP	Cromwell, 1992
Canola meal	21	MSP	NRC, 1998
Canola meal	21	MSP	Cromwell, 1992
Sorghum	20	MSP	NRC, 1998
Sorghum	20	MSP	Cromwell, 1992
Meat and bone meal	67	MSP	Cromwell, 1992
Meat and bone meal	90	MSP	NRC, 1998

[a]Bioavailability is expressed as a percentage relative to the standard which is assumed to have a bioavailability of 100%.

[b]If no standard is listed then bioavailability is expressed as a percentage of apparent absorption.

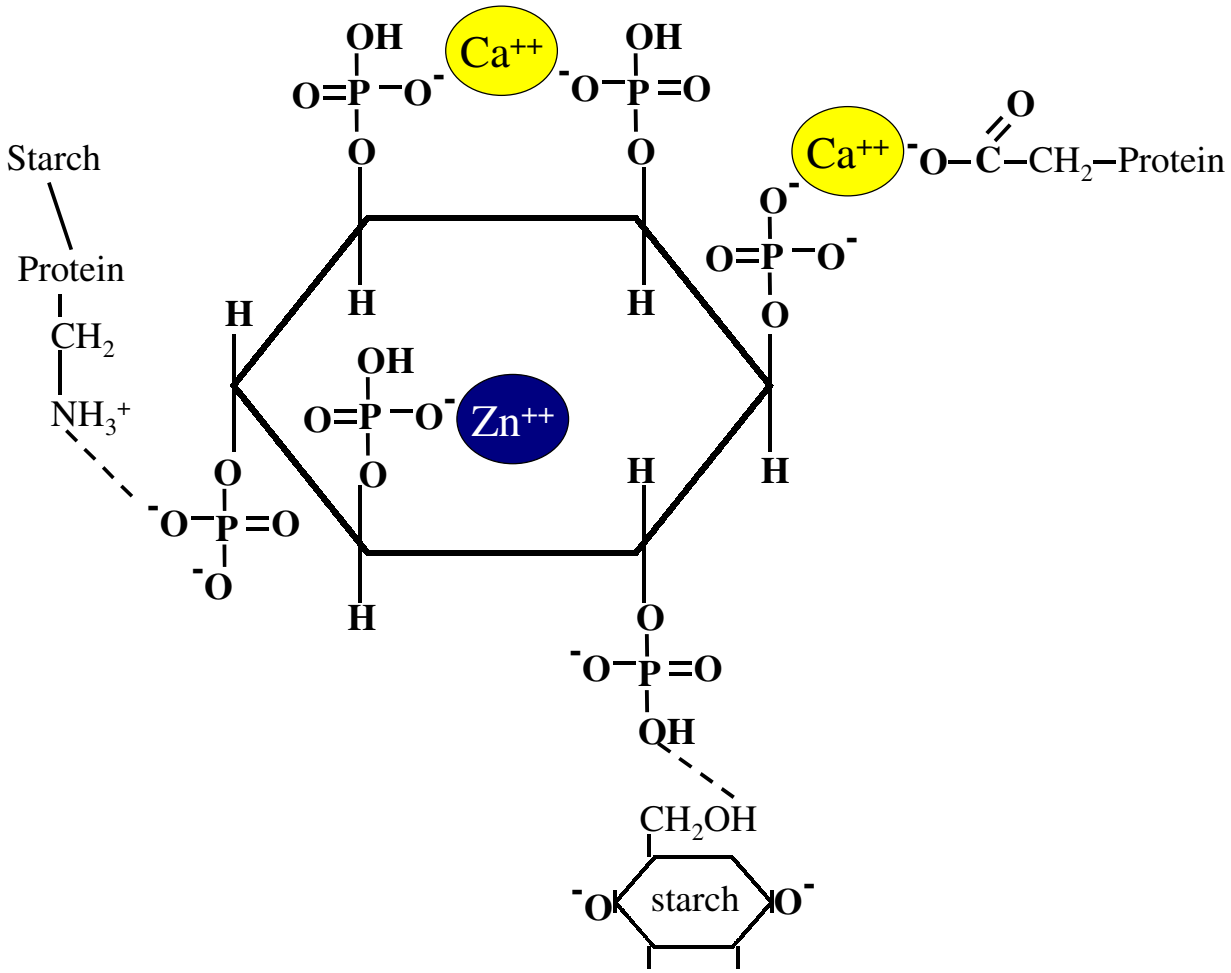


Figure 4. Mineral, amino acid, and starch chelating ability of phytic acid.

Phytic acid has the greatest affinity for Zn and Cu and a fairly low affinity for Ca. However, due to the much larger concentration of Ca in diets fed to pigs relative to Zn and Cu, an effect on Ca is possible. It has also been shown that the anionic phosphate groups of phytate possess the ability to bind proteins (Prattley et al., 1982) and amino acids; having its greatest affinity for the basic amino acids: lysine, arginine, and histidine (Reddy et al., 1982).

Phytase

Phytases are a subset of a larger enzyme group known as phosphatases (Kies, 1996). Phosphatases are enzymes which catalyze the hydrolysis of ester-linked phosphates. In this process the bound phosphate is removed from the substrate and in an intermediary step becomes bound to the enzyme before becoming dissolved in water. Specifically, phytases are phosphatases which catalyze the hydrolysis of phosphate from phytate.

Two functionally distinct phytases have been characterized to date. Both produce similar outcomes, but have different modes of action (Kies, 1996). The first, 6-phytase, catalyzes the stepwise hydrolysis of phosphate from phytate beginning at position 6 and is primarily found in plants. The second, 3-phytase, is produced by many microorganisms and catalyzes the stepwise hydrolysis of phosphate from phytate beginning at position 3 (Kies, 1996). The hydrolysis of phosphate from the phytate molecule results. In other words, 3-phytase begins hydrolyzing phosphate from phytate at position 3 and then continues at positions 4, 5, 6 and 1, producing inositol phosphate-5 (IP-5), IP-4, IP-3, IP-2, and IP-1, respectively (Venekamp et al., 1995). The 3-phytase, does not or only very slowly catalyzes the hydrolysis of IP-1 to IP plus free phosphate (Kies, 1996).

Plant Phytases. Plant phytases (6-phytase) express maximum activity at approximately pH 5.0 with values reported for barley, bean, corn, peanut, wheat, and wheat bran phytase of pH 5.2, 5.3, 5.6, 5.0, 5.2, and 5.0, respectively. Nayini and Markakis (1986) reported that the optimal pH for soybean phytase occured between pH 4.5 and 4.8. Peak plant phytase activity has been shown to occur at a temperature of 50° C with a range reported in the literature of 45 to 57° C (Irving, 1980). The amount of phytase found in plant sources varies greatly, with rye, triticale, and wheat containing the largest amounts (Table 6).

Natuphos is a commercially available phytase supplement produced by Gist-Brocades (Delft, The Netherlands) and marketed by BASF (Mount Olive, NJ). It is a 3-phytase produced by over expression of the *Aspergillus niger* phytase gene, *phyA*, in *Aspergillus niger* var. van Tieghem grown in a low phosphate medium. Microbial phytase has been shown to have two optimal pH peaks of activity at pH 2.5 and pH 5.0 to 5.5 (Shieh et al., 1969; Irving and Cosgrove, 1974; Simons et al., 1990). Simons et al. (1990), using microbial phytase from a crude preparation of *Aspergillus*, found that the phytase was 50% more active at pH 2.5, compared to pH 4.5. However, Beudeker (1990) found that Natuphos phytase was more active at pH 5.5 compared to pH 2.5. At a pH of 7.0 or higher, no activity of microbial phytase has been seen (Simons et al., 1990). The pH of a corn-soybean meal based diet using dicalcium phosphate as the P source is about 6.0.

Alltech, Inc. (Nicholasville, KY) produces and markets a phytase product in the United States. The product, Allzyme, is not genetically modified, but is produced by *Aspergillus niger* fungi. Because Alltech's product relies on crude extraction of the phytase enzyme, it contains cellulase, pectinase, xylanase, and acid phosphatase activity in addition to phytase activity.

Table 6. Intrinsic phytase activity in various feedstuffs.

Feedstuff	Phytase, U/kg	Reference
Rye	4900	Pointillart, 1994
Rye	4132-6127	Eeckhout and De Paepe, 1994
Triticale	1500	Pointillart, 1994
Triticale	1475-2039	Eeckhout and De Paepe, 1994
Wheat	700	Pointillart, 1994
Wheat	915-1581	Eeckhout and De Paepe, 1994
Barley	400	Pointillart, 1994
Barley	408-882	Eeckhout and De Paepe, 1994
Wheat Bran	1200	Pointillart, 1994
Wheat Bran	1180-5208	Eeckhout and De Paepe, 1994
Corn	0-46	Eeckhout and De Paepe, 1994
Soybeans (heated)	0-188	Eeckhout and De Paepe, 1994
SBM, 44%	0-120	Eeckhout and De Paepe, 1994
SBM, 48%	0-20	Eeckhout and De Paepe, 1994
SBM	Non-detectable	Pointillart, 1994
Canola meal	100	Pointillart, 1994
Canola meal	16	Eeckhout and De Paepe, 1994
Sorghum	24	Eeckhout and De Paepe, 1994
Wheat middlings	4381	Eeckhout and De Paepe, 1994
Wheat middlings	1900	Pointillart, 1994

Novo Nordisk (2880 Bagsvaerd, Denmark), produces and markets a genetically modified product in Europe, similar to the product marketed by BASF, but patent laws have prevented marketing of this product in the United States.

Site of Phytase Activity. Jongbloed et al. (1992) reported that 52% of the phytate P was degraded in the stomach of pigs, and an additional 9% in the duodenum and jejunum. In addition, no phytase activity could be detected in the ileum. In two experiments, Yi et al. (1996) reported that phytase activity in the digesta decreased from the stomach to the upper small intestine to the lower small intestine when measured 3 h after ingestion of a meal. Phytase activity, as a percentage of the total dietary phytase activity, was found to be 51% in the stomach, 31% in the upper small intestine, and 5% in the lower small intestine in experiment 1. In experiment 2 values of 41%, 16%, and 5% were reported for the stomach, upper small intestine, and lower small intestine, respectively. The acidity of the stomach lumen ranges from pH 1.0 to 4.5 (Chessen, 1987) and the luminal pH of the gastrointestinal tract increases from the duodenum to the terminal ileum. Jongbloed et al. (1992) reported that the duodenal pH immediately following a meal was 5.7, after which it gradually decreased to pH 3.3. It is generally accepted that the duodenal pH is approximately 4.8. The jejunum, which represents the largest segment of the small intestine (approximately 90% of the total length), has a mean pH of 5.5 to 6.9 and the ileum has a mean pH of 7.0 to 7.4

Phytase Effects on P Digestibility. Approximately, 60 to 70% of the P found in plant ingredients commonly used for pigs is bound as phytate P, and is therefore unavailable for absorption (Cromwell, 1992; Ravindran et al., 1994, 1995). Nelson et al. (1968) demonstrated the ability of phytase to release this bound P. However, it has not been until recently that a commercially available phytase preparation was approved for use in swine diets. Natuphos

phytase has been approved for use in Europe since 1991, and was approved for use in the U. S. on November 17, 1995. Addition of microbial phytase has been shown to catalyze the hydrolysis of the phytate molecule, releasing the bound P (Jongbloed et al., 1992; Cromwell et al., 1993; Lei et al., 1993b, Kornegay et al., 1995, 1996; Jongbloed, 1996; Radcliffe and Kornegay, 1998; Skaggs, 1999; Rice et al., 1999; Zhang, 1999; Rice et al., 2000; Robbins et al., 2000).

Microbial phytase has been shown to affect performance of pigs fed low P diets by increasing average daily gains (Simons et al., 1990; Beers and Jongbloed, 1992; Jongbloed et al., 1992; Kornegay and Qian, 1996; Yi et al., 1996c) primarily due to an increased feed intake (Simons et al., 1990; Beers and Jongbloed, 1992; Jongbloed et al., 1992; Kornegay and Qian, 1996; Yi et al., 1996c). Increases in bone breaking strength or shear force have also been demonstrated in several studies with pigs (Cromwell et al., 1993; Ketaert et al., 1993; Kornegay and Qian, 1996; Yi et al., 1996c; Radcliffe and Kornegay, 1998). The addition of microbial phytase also decreases P excretion 25 to 50% (Simons et al., 1990; Jongbloed et al., 1992; Cromwell et al., 1993a; Lei et al, 1993b; Kornegay and Qian, 1996; Yi et al., 1996c) by increasing P digestibility or retention (Hoppe et al., 1992; Lei et al, 1993a,b; Mroz et al., 1994; Kornegay and Qian, 1996; Yi et al., 1996c).

Similar results to those observed in pigs have been demonstrated in broilers when microbial phytase is supplemented to low P diets. An increased BW gain in broilers observed when phytase is added (Denbow et al., 1995; Qian et al., 1996; Yi et al., 1996a,d) can be attributed primarily to an increased feed intake as the level of phytase in the diet was increased (Denbow et al., 1995; Qian et al., 1996; Yi et al., 1996a,d). Increases in tibia shear force (Denbow et al., 1995; Qian et al., 1996) and toe (Denbow et al., 1995; Yi et al., 1996a,d) or tibia ash (Qian et al., 1996; Yi et al., 1996d) have also been observed in broilers fed diets

supplemented with microbial phytase. Yi et al. (1996d) reported a decreased P excretion in phytase supplemented broilers which could be attributed to increased P retention (Schöner and Hoppe, 1992; Yi et al., 1996d).

Phosphorus Equivalency Values of Phytase. In order for farmers to efficiently use microbial phytase as a supplement, accurate equivalency values of phytase for P must be developed. Ideally, in studies designed to develop equivalency values, multiple levels of P should be fed without added phytase and multiple levels of phytase at a low level of P in order to develop response equations for both P and phytase. These response equations can then be set equal to one another to determine the P equivalency of phytase. In general, linear ($Y = a + bX$; where Y = response and X = the level of P or phytase) or asymptotic ($Y = a(1 - be^{-kX}$; where Y = response and X = the level of P or phytase) curves have provided the best fits for phytase and P responses in corn-soybean meal based diets (Kornegay et al., 1998). Jongbloed et al. (1996) reported that a logistic curve provided a better fit to the response of P and phytase in a Dutch practical diet.

Before examining the results of several studies attempting to generate P equivalency values, it is first necessary to distinguish between the amount of phytate P released by phytase and P equivalency values of phytase for inorganic P. Many studies in the literature do not attempt to distinguish between these, but they are quite different. The amount of phytate P released by phytate refers to the amount of P released due to the hydrolysis of phytate P. This value is lower than the P equivalency value because phytase is replacing inorganic P which is less than 100% available. For example, if 500 U of phytase per kilogram of diet could catalyze the release of 0.76 g of phytate P, then the P equivalency value would be equal to 0.76 divided by the percent availability of the inorganic P source. If the P from inorganic P was 75% available

then the P equivalency value of phytase would be 1.01 g of P from inorganic P (0.76/0.75). Therefore, in order to determine the amount of inorganic P that can be replaced in a given diet, it is essential to know the bioavailability of the inorganic P source. The bioavailability of P from inorganic P sources used in swine diets is generally considered to be quite high. However, there has been a substantial amount of variation reported in the literature. Soares (1995) suggested a relative bioavailability value of 90% for defluorinated phosphate, 95% for dicalcium phosphate and 100% for monocalcium phosphate when monosodium phosphate was used as the standard and given a value of 100. A relative bioavailability of 87% is suggested for defluorinated phosphate and a value of 100% is suggested for dicalcium phosphate when monocalcium phosphate is given a value of 100 (NRC, 1998). Kornegay and Radcliffe (1997) compared four sources of defluorinated phosphate and one source of dicalcium phosphate against a monocalcium phosphate control. They found no differences between any of the phosphate sources. The relative bioavailabilities ranged from 95.1 to 105.3%. The availability of each P source in this study can be estimated by dividing the increase (digested P in diet - basal digested P) in digested P (g/kg) when P is added by the amount of added inorganic P added and multiplying by 100. With the exception of one of the defluroinated phosphate sources (bioavailability = 58.5%) the estimated bioavailabilities of P from all sources used in the study by Kornegay and Radcliffe (1997) ranged from 71.9 to 79.3 %, with the average being 75.1%. This is in good agreement with a recent review by Kornegay et al. (1998) in which the estimated bioavailability of inorganic P across 52 experiments was 76.7% for swine.

In several studies attempts have been made to determine the P equivalency value of phytase in broilers (Table 7; Denbow et al., 1995; Ravindran et al., 1995; Yi et al., 1996d) and in

Table 7. Phosphorus equivalency equations reported in the literature for broilers.

Item	Equation[a]	Released P (g/kg)	Reference
BW gain			
SP[b]	$Y = -9.615Ln(0.9662 + 0.0153e^{-0.0037X})$	1.07	Denbow et al., 1995[c]
SP	$Y = -9.615Ln(0.9643 + 0.0101e^{-0.0057X})$	.74	Denbow et al., 1995[d]
SP	$Y = -0.112Ln(-0.058 + 0.150e^{-0.0005X})$	.47	Yi et al., 1996d
CS	$Y = -0.172Ln(0.004 + 0.206e^{-0.0011X)}$	1.02	Yi et al., 1996d
SP	$Y = -0.0559Ln(0.000713 + 0.0073e^{-0.0046X})$	.96	Ravindran et al., 1995[d]
SP	$Y = -0.0559Ln(-0.00098 + 0.00227e^{-0.0007X})$	.53	Ravindran et al., 1995[e]
Toe ash, %			
SP	$Y = -0.4587Ln(0.4538 + 0.1875e^{-0.0013X})$	.73	Denbow et al., 1995[c]
SP	$Y = -0.4587Ln(0.4419 + 0.1114e^{-0.0032X})$	.82	Denbow et al., 1995[d]
SP	$Y = -0.126Ln(0.123 - 0.00007X)$	.36	Yi et al., 1996d
CS	$Y = -0.122Ln(0.069 + 0.039e^{-1.01X})$	.56	Yi et al., 1996d
SP	$Y = -0.1745Ln(0.08854 + 0.12187e^{-0.0022X})$	.87	Ravindran et al., 1995[d]
SP	$Y = -0.1745Ln(0.06728 + 0.08505e^{-0.0056X})$	.98	Ravindran et al., 1995[e]

[a]Y = digestible P (g/kg) and X = phytase activity (U/kg).
[b]SP = semipurified diet and CS = corn-soybean meal based diet.
[c]Equations based on a basal diet containing .20% nP.
[d]Equations based on a basal diet containing .27% nP.
[e]Equations based on a basal diet containing .36% nP.

pigs (Table 8; Kornegay and Qian, 1996, Jongbloed et al., 1996; Yi et al., 1996c; Harper et al., 1997; Radcliffe and Kornegay, 1998; Rice et al., 1999; Skaggs, 1999).

The P equivalency value for broilers for 500 U of phytase per kilogram of diet ranges from 0.207 g to 0.458 g. In pigs the range of equivalency values for 500 U/kg phytase is much larger ranging from 0.64 g P to 2.47 g P. Factors which may influence these equivalency value estimates include: the basal level of P, the response criteria used, and perhaps most importantly the ratio of Ca to P. Phosphorus absorption has been shown to be impaired if the Ca:P ratio is too wide (NRC, 1998). In addition, Qian et al. (1996a) reported a detrimental effect of a widening Ca:P ratio in excess of 1.2:1 on phytase efficacy in pigs. In a similar study with broilers, Qian et al. (1996b) reported that widening the Ca:P ratio from 1.4:1 to 2.0:1 decreased the efficacy of microbial phytase. Excess Ca may bind to the phytate molecule, making it insoluble and therefore unavailable for exposure to phytase in the gastrointestinal tract. In the studies investigating the equivalency value of microbial phytase for P in broilers which were reported above (Denbow et al., 1995; Ravindran et al., 1995; Yi et al., 1996d), the Ca:P ratio in all cases was 2:1 except for the positive control diet in the study by Ravindran et al. (1995) where the Ca:P ratio was 1.46:1. In the pig studies of Kornegay and Qian (1996), Jongbloed et al. (1996), and Yi et al. (1996c) only two levels of P were fed, and the response of various criteria to P was assumed to be linear. In addition, the Ca:P ratio in the studies of Kornegay and Qian (1996) and Yi et al. (1996c) was 2:1. In the study by Jongbloed et al. (1996) the Ca:P ratio ranged from 1.94:1 to 2.5:1. In a study with growing-finishing pigs, Harper et al. (1997), utilized three levels of P and maintained a Ca:P ratio of approximately 1.2:1 to 1.4:1 in all diets. They reported that on average 500 U of microbial phytase released .96 g of P per kilogram of diet.

Table 8. Phosphorus equivalency equations reported in the literature for pigs.

Parameter	Diet[a]	Equation[b]	Equivalency (g/kg)	Reference
ADG				
	SP	$Y = 3.41 - 3.07e^{-.0003X}$	.80	Yi et al., 1996c[c]
	SP	$Y = 1.68 - 2.17e^{-.0016X}$	.70	Yi et al., 1996c[d]
	CS	$Y = 4.062 - 3.865e^{-0.00095X}$	1.66	Kornegay and Qian, 1996[e]
	CS	$Y = 3.362 - 3.380e^{-0.00266X}$	2.47	Kornegay and Qian, 1996[d]
	CS	$Y = 0.0654 - 0.0741e^{-0.00839X}$	.64	Harper et al., 1997
	CS	$Y = 0.084 + 0.002X$	.99	Radcliffe and Kornegay, 1998
	CS	$Y = 1.19 - 1.25e^{-0.0050X}$	1.08	Radcliffe and Kornegay, 1998
	CS	$Y = .277 - .274e^{-0.000797X}$	.93	Skaggs, 1999
	CS	$Y = .0977 - .0988e^{-0.0035X}$	.81	Skaggs, 1999
	CS	$Y = .176 + .00213X$	1.24	Rice et al., 1999
	CS	$Y = .033 + .0032X$	1.63	Rice et al., 1999
10^{th} rib ash, %				
	SP	$Y = 1.03 - 1.00e^{-.0015X}$	.56	Yi et al., 1996c[c]
	SP	$Y = 1.06 - 1.09e^{-0.0014X}$	.52	Yi et al., 1996c[d]
	CS	$Y = 1.848 - 1.926e^{-0.0045X}$	1.65	Kornegay and Qian, 1996[e]
	CS	$Y = 1.629 - 1.806e^{-0.0036X}$	1.33	Kornegay and Qian, 1996[d]
10^{th} rib ash weight				
	CS	$Y = 0.0102 + .0015X$	.76	Radcliffe and Kornegay, 1998
	CS	$Y = 0.8699 + 0.9413e^{-0.0036X}$	1.02	Radcliffe and Kornegay, 1998
10^{th} rib shear force				
	CS	$Y = 0.348 - 0.357e^{-0.00082X}$	1.11	Harper et al., 1997
	CS	$Y = -0.0243 + 0.0014X$	.69	Radcliffe and Kornegay, 1998
Metacarpal ash, %				
	CS	$Y = .112 - .1118e^{-.0029X}$	.85	Skaggs, 1999

Table 8. (Continued) Phosphorus equivalency equations reported in the literature for pigs.

Parameter	Diet[a]	Equation[b]	Equivalency (g/kg)	Reference
	CS	$Y = .1127 - .1184e^{-0.00095X}$	.39	Skaggs, 1999
Metacarpal shear force				
	CS	$Y = .0057 + .000005X$	.27	Skaggs, 1999
	CS	$Y = .0862 - .0891e^{-0.0014X}$	.41	Skaggs, 1999
	CS	$Y = .00097 + .00149X$	.75	Rice et al., 1999
	CS	$Y = -.0788 + .002X$	.93	Rice et al., 1999
Digestible P				
	CS	$Y = 1.01 - 1.0013 \times 0.9963^{X}$	.85	Jongbloed et al., 1996
	Dutch	$Y = -0.1786 + 1.31/(1 + e^{(-0.0051 \times (X - 378))})$	.67	Jongbloed et al., 1996
	CS	$Y = .173 - .177e^{-0.00102X}$	.67	Skaggs, 1999
	CS	$Y = .0657 - .0596e^{-0.0019X}$	.42	Skaggs, 1999
P digestibility, %				
	SP	$Y = 1.30 - 1.21e^{-.0019X}$	.83	Yi et al., 1996c[c]
	SP	$Y = 1.31 - 1.51e^{-.0036X}$	1.10	Yi et al., 1996c[d]
	CS	$Y = 2.631 - 2.965e^{-0.00108X}$	1.19	Kornegay and Qian, 1996[e]
	CS	$Y = 1.564 - 1.735e^{-0.00284X}$	1.14	Kornegay and Qian, 1996[d]
	CS	$Y = -0.087Ln(-6.718 + 7.713e^{-0.000199X})$	1.16	Harper et al., 1997
	CS	$Y = -0.464Ln(0.888 - 0.0014X)$	.78	Radcliffe and Kornegay, 1998
	MIX	$Y = .7452 - .4280$	.63	Kornegay et al., 1998
	CS	$Y = .1552 - .1489e^{-0.00198X}$	.99	Skaggs, 1999
	CS	$Y = -.112 + .0037X$	1.76	Rice et al., 1999
	CS	$Y = -.22 + .0038X$	1.67	Rice et al., 1999

[a]SP = semipurified diet, CS = corn-soybean meal based diet, and Dutch = Dutch practical diet.
[b]Y = digestible P (g/kg) and X = phytase activity (U/kg)
[c]Equations based on a basal diet containing .05% aP.
[d]Equations based on a basal diet containing .16% aP.
[e]Equations based on a basal diet containing .07% nP.

Radcliffe and Kornegay (1998) fed 96 crossbred weanling pigs a low P (3.5 g/kg) diet with supplemental inorganic P (0, 0.5, 1.0, or 1.5 g/kg) or microbial phytase (0, 167, 333, or 500) to determine the P equivalency value of microbial phytase. They found that the addition of microbial phytase to the low P diet improved ADG, rib shear force, shear energy and ash (% and weight), and Ca and P digestibility. The addition of P to the low P diet improved ADG, rib shear force, energy and ash (% and weight), and Ca and P digestibility. After regressing response criteria against P or phytase level, linear ($Y = mX + b$) P response curves provided good fits for P digestibility and rib shear force and non-linear ($Y = a(1-be^{-kX})$) P response curves provided good fits for ADG and rib ash weight. Phytase addition resulted in linear responses for ADG and rib ash weight and shear force. Non-linear responses were observed for P digestibility. By setting the P response equations equal to the phytase response equations and solving for P, phytase equivalency values were derived. In that study 500 U/kg of phytase was equivalent to .84 g of P as inorganic P.

Kornegay et al. (1998) used data from 52 pig experiments to estimate the P equivalency value of phytase. They used data from these experiments to generate response curves for P digestibility (%), digested P (g/kg), and P excretion as influenced by phytase and P. Figure 5 shows the phytase response curve that was developed for P digestibility. Phytase unquestionably increases P digestibility. However, the magnitude of this response is dependent on diet type, total P content of the diet, phytate P content of the diet, Ca:P ratio, and the age and physiological status of the pig. These criteria account for the wide range of P digestibilities reported in the literature. The P digestibility curve described for phytase by Kornegay et al. (1998) was plotted with the actual data used to generate the curve on the same graph in Figure 5. Examining the basal diets, with no added phytase, a range of P digestibility of 8.4 to 63.0% was observed. This

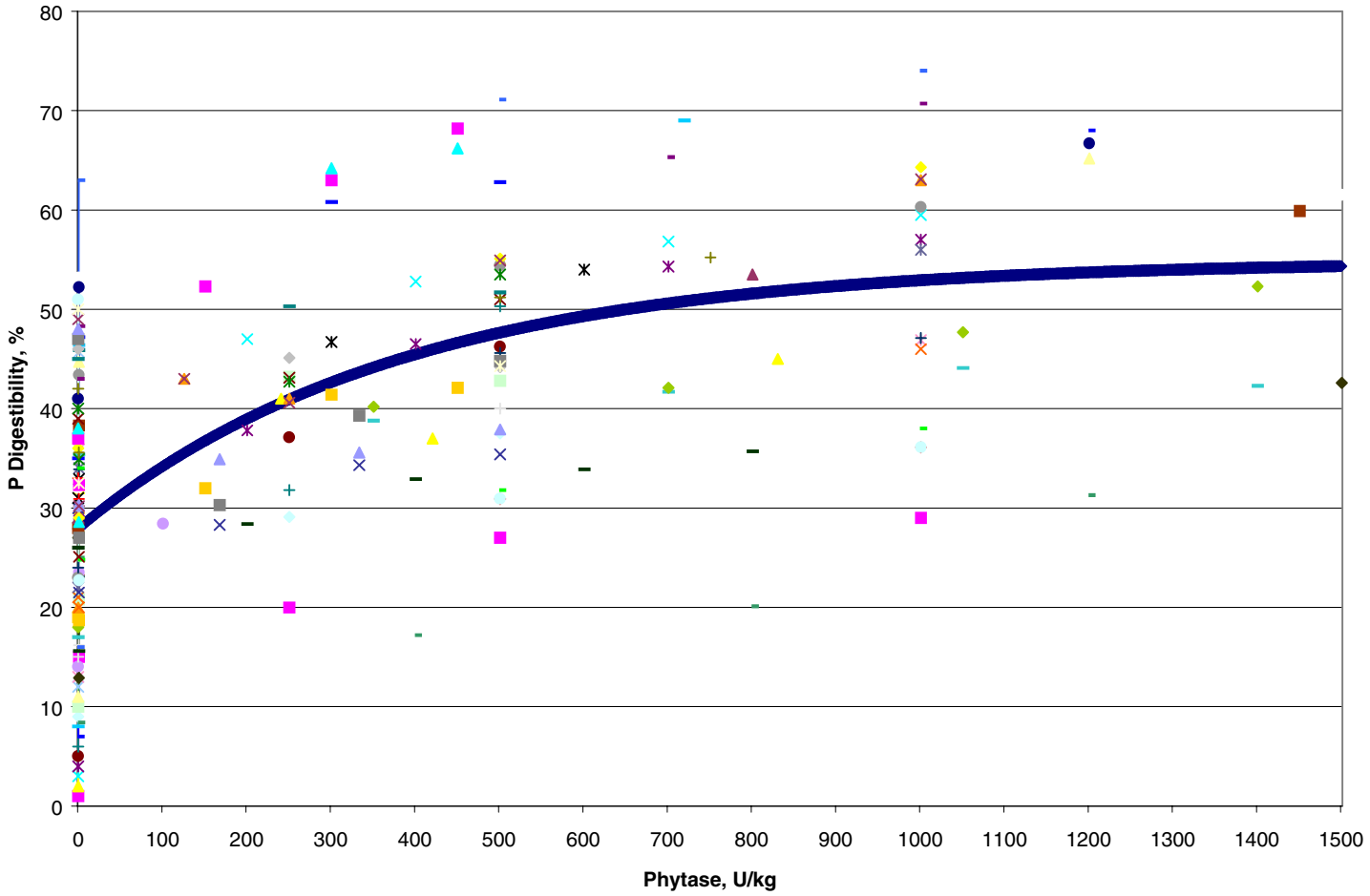


Figure 5. P digestibility response curve adapted from Kornegay et al. (1998). Data used to derive the response curve were obtained from: Adeola and Sutton, 1994; Beers et al., 1992; Cromwell et al., 1995; Eeckhout and de Paepe 1991; Han et al., 1997, Harper and Kornegay 1997; Jongbloed et al., 1992, 1996; Kornegay and Qian 1996; Kornegay, 1998a; Lei et al., 1993a,b; Liu et al., 1997; Mroz et al., 1993,1994; Murry et al., 1997; Näsi, 1991.

wide range is due to the inclusion of plant feedstuffs with intrinsic phytase activity in some studies, differences in the Ca to P ratio, differences in the inclusion level of inorganic P, and differences in the phytate P level of the diet. This variation continues as phytase is added to the diet and causes the relatively poor fit (r^2 = .47) of the response curve calculated in that review. However, if the equation and the observed values are adjusted by calculating the percentage unit improvement in P digestibility as phytase is added to the diet, then the variation is decreased (Figure 6). If the improvement in P digestibility curve extrapolated from the work of Kornegay et al. (1998) is plotted with the improvement in P digestibility curve generated in an early review by Dungelhoef and Rodehutschord (1995) there is very good agreement between the two curves (Figure 7). The curves predict almost identical values up to 800 U of added phytase per kilogram of diet. Jongbloed (1996) took data from an earlier experiment (Beers and Jongbloed, 1992) and fit a P digested (g/kg) curve. By taking these numbers, back calculating digestibility values, and subtracting out the basal digestibility (27.5%) of P, it is possible to develop a phytase response curve for the increase in P digestibility. Plotting this against the curves of Kornegay et al. (1998) and Dungelhoef and Rodhutschord (1995) show a higher estimation of P digestibility improvement with added phytase by Jongbloed (1996). To adjust for differences in the basal level of dietary P, the phytase response curves for the increase in P digested (g/kg) were plotted (Figure 7). For Jongbloed (1996) and Dungelhoef and Rodehutschord (1995) the equations describing these curves were taken from their respective papers and used to develop the response curves. For Kornegay et al. (1998) the increase in digested P (g/kg) was calculated as the increase in P digestiblity (%) times the average amount of P in the diets (3.8 g/kg). The basal level of P was then subtracted from these values to provide values describing the increase in P digested (g/kg) response curve. As shown in Figure 8, this brought all three curves in closer

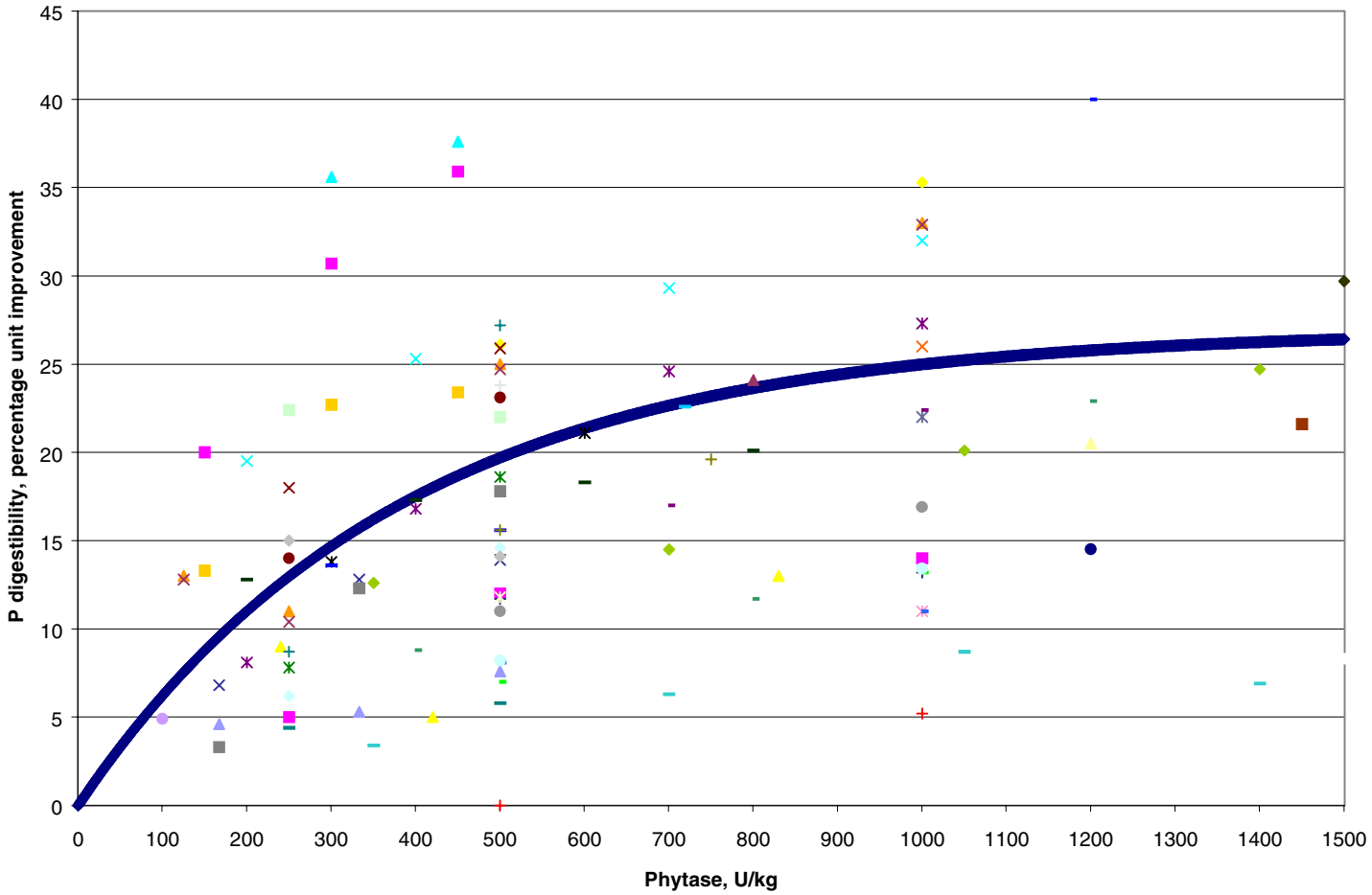


Figure 6. Increase in P digestibility (percentage unit) response curve cacluated from Kornegay et al. (1998). Data used to derive the response curve were obtained from: Adeola and Sutton, 1994; Beers et al., 1992; Cromwell et al., 1995; Eeckhout and de Paepe 1991; Han et al., 1997, Harper and Kornegay 1997; Jongbloed et al., 1992, 1996; Kornegay and Qian 1996; Kornegay, 1998a; Lei et al., 1993a,b; Liu et al., 1997; Mroz et al., 1993,1994; Murry et al., 1997; Näsi, 1991.

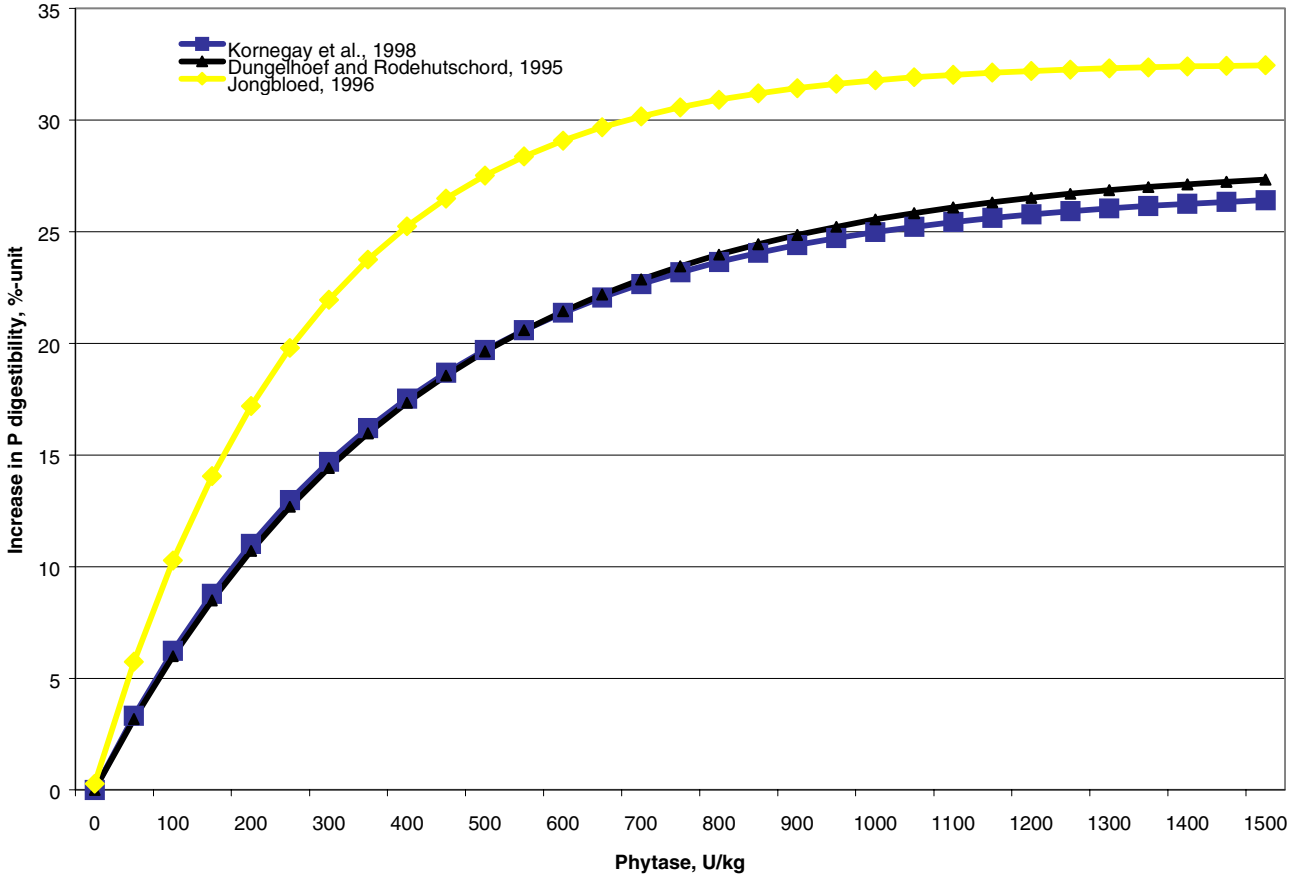


Figure 7. Comparison of phytase response curves for the increase in P digestibility (percentage unit). The response curve of Kornegay et al., was based off of the P digestibility response curve which they presented. Their response curve was generated with data from: Adeola and Sutton, 1994; Beers et al., 1992; Cromwell et al., 1995; Eeckhout and de Paepe 1991; Han et al., 1997, Harper et al., 1997; Jongbloed et al., 1992, 1996; Kornegay and Qian 1996; Kornegay, 1998a; Lei et al., 1993a,b; Liu et al., 1997; Mroz et al., 1993,1994; Murry et al., 1997; Näsi, 1991. The response curve of Dungelhoef and Rodehutschord was based off of data from: Näsi 1990; Simons et al 1990, Borggreve et al. 1991; Beers 1992; Beers et al., 1992; Eeckhout and De Paepe 1992a,b,c; Jongbloed et al. 1992; Lantzsch and Wjst 1992; Jongbloed et al. 1993; Kemme and Jongbloed 1993; Mroz et al. 1993, 1994; Kemme and Jongbloed 1994. The response curve of Jongbloed (1996) was based on data from: Beers and Jongbloed, 1992.

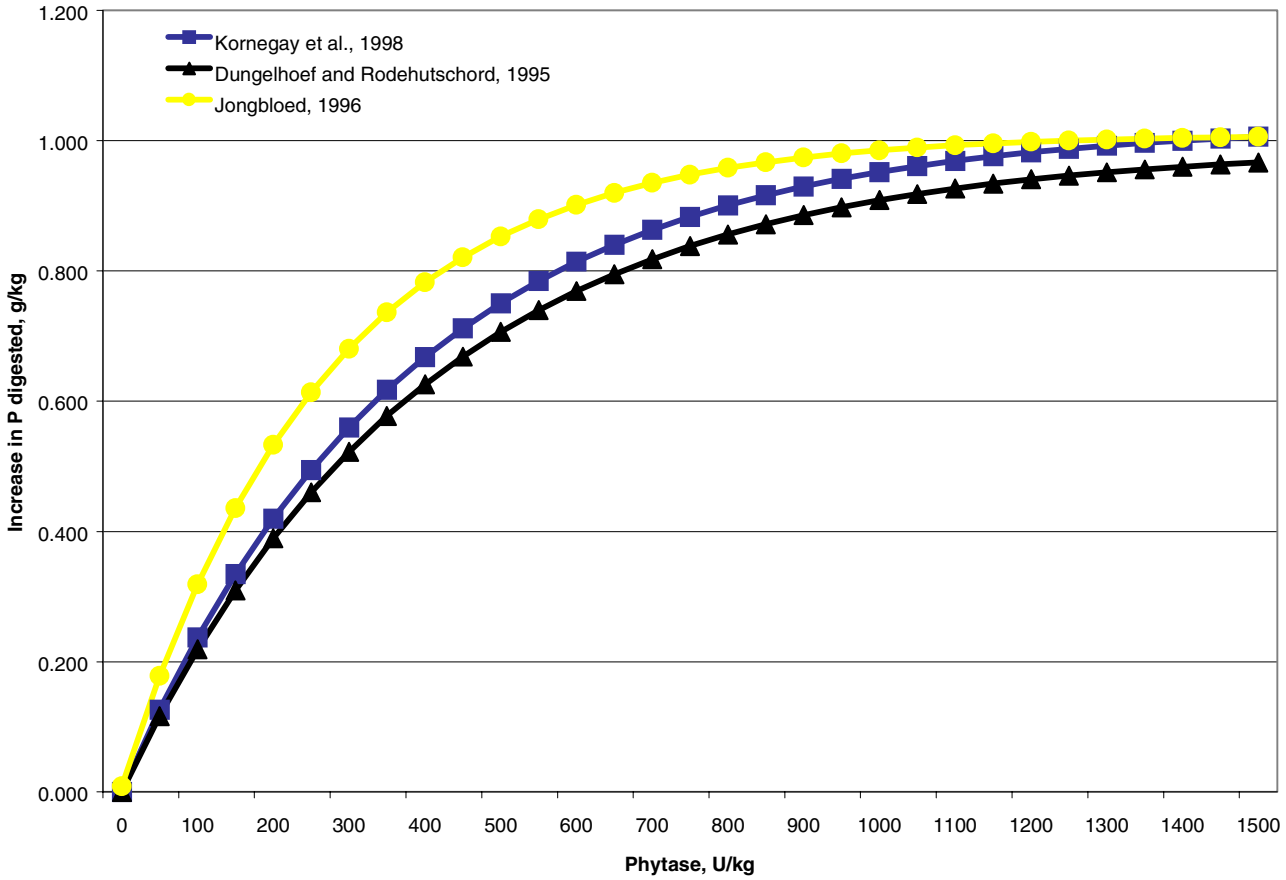


Figure 8. Comparison of phytase response curves for the increase in P digested (g/kg of diet). The response curve of Kornegay et al., was based off of the P digestibility response curve which they presented. Their response curve was generated with data from: Adeola and Sutton, 1994; Beers et al., 1992; Cromwell et al., 1995; Eeckhout and de Paepe 1991; Han et al., 1997, Harper et al., 1997; Jongbloed et al., 1992, 1996; Kornegay and Qian 1996; Kornegay, 1998a; Lei et al., 1993a,b; Liu et al., 1997; Mroz et al., 1993,1994; Murry et al., 1997; Näsi, 1991. The response curve of Dungelhoef and Rodehutschord was based off of data from: Näsi 1990; Simons et al 1990, Borggreve et al. 1991; Beers 1992; Beers et al., 1992; Eeckhout and De Paepe 1992a,b,c; Jongbloed et al. 1992; Lantzsch and Wjst 1992; Jongbloed et al. 1993; Kemme and Jongbloed 1993; Mroz et al. 1993, 1994; Kemme and Jongbloed 1994. The response curve of Jongbloed (1996) was based on data from: Beers and Jongbloed, 1992.

agreement with each other. The equations used to generate the curves for Figures 6 to 8 are shown in Table 9. Based on the review by Kornegay et al. (1998) 500 U of phytase per kg of diet will release .75 g of P. If this number is divided by the estimated bioavailability of inorganic P (76.7%) then 500 U of phytase per kg of diet can replace .98 g of P from an inorganic P source. This is in good agreement with the findings of Harper et al. (1997) and Radcliffe and Kornegay (1998).

Phytase Effects on Calcium. Phytic acid, at a neutral pH carries one or two negatively charged oxygen atoms per phosphate group (Erdman, 1979). Therefore, each molecule of phytase carries 6 to 12 negative charges at or near a neutral pH, like that found in the jejunum and ileum. Therefore, one mole of phytic acid can bind an average of 3 to 6 moles of Ca to form insoluble phytates at the pH of the small intestine. This binding potentially renders Ca unavailable for intestinal absorption. If a 19% crude protein corn-soybean meal diet contains .1118% or 27.95 mmoles (1,118/40) of Ca, and .27% phytate P (.924% phytate) or 14.3 mmoles (9,240/648) of phytic acid, the ratio of Ca:phytic acid would be 1.95:1. Theoretically, all of the Ca in the plant ingredients could be bound, and perhaps some of the supplemental Ca. Addition of microbial phytase to the diet results in the stepwise hydrolysis of PO_4 from the inositol ring of phytate (Venekamp et al., 1995; Kies, 1996). This prevents the formation of cation-phytate salts in the small intestine.

Calcium was first reported to be associated with phytate by McCance and Widdowson in 1935 (Oberleas and Harland, 1996). Nelson et al. (1968) showed that as the level of phytic acid in the diet increased so did the chick's requirement for Ca and that this effect decreased when phytase was added to the diet. Eeckhout and De Paepe (1991) reported a high positive correlation between Ca and P digestibilities when phytase was added to a low P pig diet. They

Table 9. Equations used to generate phytase response curves in figures 6-8 and response values for 250, 500, and 1,000 U of added phytase activity per kg of diet as predicted by the equations used to develop the curves.

Equations for parameters	Response to phytase, U added per kg of diet			Reference
	250	500	1,000	
P digestibility, %				
$Y = 54.86(1-0.4908e^{-0.00263X})$	40.91	47.63	52.92	Kornegay, et al., 1998
P Digestibility, %-unit				
$Y = 26.93(1-e^{(-0.00263X)})$	12.98	19.70	24.99	Kornegay, et al., 1998
$Y = 28.1(1-e^{-0.0024X})$	12.68	19.64	25.55	Dungelhoef and Rodehutschord, 1995
$Y = (59.197 - 32.30*0.9963^{X})-27.5$	19.79	27.52	31.79	Jongbloed, 1996
Increase in digested P, g/kg				
$Y = 1.026(1-e^{-0.00263X})$	.494	.751	.952	Kornegay, et al., 1998
$Y = 0.99(1-e^{-0.0025X})$	.460	.706	.909	Dungelhoef and Rodehutschord, 1995
$Y = 1.01-(1.0013*0.9963^{X})$	.614	.853	.985	Jongbloed, 1996

suggested that this relationship could be explained by the fact that phytic acid acts as a Ca binding agent in the proximal small intestine. Therefore, hydrolysis of phytate in the stomach as a result of phytase activity results in increased digestibility, not only of P, but indirectly of Ca. Pointillart (1993) also found in his studies, primarily with cereal phytase, that improved P utilization was generally accompanied by improved Ca retention.

Improved Ca retention of broilers has been reported when supplemental phytase was added to the diet (Schöner et al., 1991, 1993; Kornegay et al., 1996; Yi et al., 1996d). Mroz et al. (1993) reported enhanced Ca and P digestibility in 30 kg pigs when 300 and 600 U of phytase/kg were added to a basal diet containing suboptimal levels of Ca (.43%) and tP (.43%). Hypophosphaturia and hypercalciuria developed in pigs fed the basal diet, which was prevented by adding .05% P from KH_2PO_4 or 300 U of phytase per kilogram of feed. Body weight gain and gain:feed ratios were similar among treatments. Based on this limited number of studies, it does appear that phytase has the potential to make more dietary Ca available for utilization by the pig. In order for swine producers to feed optimal and not excessive levels of Ca and P and in order to maintain an optimal Ca to P ratio in swine diets, equivalency values of phytase for Ca must be developed.

Radcliffe (1997) reported on two experiments in which weanling pigs were fed multiple levels of phytase and Ca so that response equations could be derived for various criteria and used to calculate equivalency estimates of phytase for Ca. The average equivalency estimate was 1.08 g Ca for 500 U of phytase in Exp. 1 when limestone was used and .78 g Ca for 500 U of phytase in Exp. 2 when $CaCO_3$ was used (Table 10). The difference in equivalency estimates between these studies may be due to several factors including: the basal Ca level, the Ca source, and the Ca:P ratio.

Table 10. Response equations for Ca and phytase.

	Phytase (X_1)	r^2	Calcium (X_2)	r^2
Radcliffe, 1997-Experiment 1				
ADG, wk 3-4, kg	$=.516 + .000093X_1$	.98	$=.432 + .0256X_2$	.91
Ash, %	$=45.4 + .00319X_1$	.99	$=37.9 + 1.806X_2$	.82
DCa Fecal, %	$=.296 - .03629e^{-.0038X1}$	.82	$=.855 - 1.1173e^{-.158X2}$	.99
Radcliffe, 1997-Experiment 2				
ADG, wk 3-4, kg	$=.5916 + .000149X_1$	.99	$=.6699 - 14.3694e^{-2.04X2}$	.85
Ash Wt., g	$=.8560 + .0004X_1$	.75	$=.3265 + .2166X_2$	.79
Force, N	$=669.92 - 153.41e^{-.0016X1}$	.99	$=685.94 - 22814.36e^{-1.885X2}$	.93
DCa Fecal, %	$=.2158 + .000042X_1$	.97	$=.02649 + .07097X_2$	.91
DCa Ileal, %	$=.2105 - .0412e^{-.0054x1}$	.99	$=.0125 + .0566X_2$	.89

The apparent total tract digestibility of Ca from $CaCO_3$ and limestone was estimated to be 64% and 82%, respectively in the studies by Radcliffe et al. (1997). Therefore, 500 U of phytase per kilogram of diet releases between .5 (.82 x 1.08g) and .84 g (.64 x .78 g) of digestible Ca. In a review by Jongbloed et al. (1996) they suggested that the addition of 500 U/kg of microbial phytase would release .4 to .7 g of digestible Ca per kg of diet. Using turkey poults, Kornegay et al. (1996) reported an average Ca equivalency value of .87 g total Ca being equal to 500 U/kg of microbial phytase. In their study, BW gain provided the highest Ca equivalency estimate (500 U phytase = 1.2 g/kg Ca) followed by gain:feed (500 U phytase = 0.7 g/kg Ca) and digestible Ca (500 U phytase = 0.7 g/kg Ca). Schöner et al. (1994) reported that 500 U of microbial phytase was eqiuvalent to .35 g/kg of total Ca as measured by BW gain and .56 g/kg of total Ca as measured by phalanx ash. In both experiments by Radcliffe (1997), fecal digested Ca provided the lowest estimate for equivalency values (Exp. 1, .62; Exp. 2, .36). In Exp. 2, the equivalency value based on ileal digested Ca (500 U = .85 g Ca) was much higher than the one based on fecal digested Ca (500 U = .36 g Ca), suggesting that a significant amount of Ca is excreted into the lumen of the large intestine. Bone parameters (Exp. 1: ash %, 500 U = 1.32 g Ca; Exp. 2: ash wt., 500 U = .85 g Ca; shear force, 500 U = .42 g Ca) tended to give intermediary equivalency values and growth performance (Exp. 1:ADG (wk 3 to 4), 500 U = 1.30 g Ca; Exp. 2: ADG (wk 3 to 4), 500 U = 1.43 g Ca) tended to give the highest equivalency values.

Phytase Effects on Other Minerals. Phytate has been shown to bind divalent cations in the following preferential order: $Cu^{++} > Zn^{++} > Co^{++} > Mn^{++} > Mg^{++} > Fe^{++} > Ca^{++}$ (Maddaiah et al., 1964; Vohra et al., 1965). Of the trace minerals, Zn has probably received the most attention. Lei et al. (1993c) reported that when phytase was added to the diet, the bioavailability of Zn was increased. Roberson and Edwards (1994) found no effect on Zn retention when microbial

phytase was added to broiler diets. Several studies have shown improvements in Zn status when microbial phytase was supplemented to the diet of pigs (Pallauf et al., 1992; Nasi and Helander, 1994), broilers (Biehl et al., 1995; Yi et al., 1996a) and rats (Rimbach and Pallauf, 1993; Rimbach et al., 1995). Yi et al. (1996a) fed four levels of Zn (0, 5, 10, and 20 ppm) with no added phytase and four levels of phytase (150, 300, 450, and 600 U/kg) with no added Zn to broilers so that response equations to phytase and Zn could be developed. The equivalency equation of phytase for Zn was $Y = 0.20 + 0.0082X$; where Y = mg/kg of Zn and X = U/kg of phytase. Based on this equation, 100 U/kg of phytase releases 0.9 mg/kg of Zn. Pallauf et al. (1992) and Nasi and Helander (1994) both reported increases in the apparent absorption of Mg, Fe, and Cu when microbial phytase was added to pig diets.

Phytase Effect on Heavy Metals. Two major heavy metals of interest are Pb and Cd, both of which have been shown to interact with phytic acid (Wise and Gilburt; 1981; Nolan and Duffin, 1987; Rimbach et al., 1994). Cadmium has received the most attention thus far. Cadmium is a toxic element which builds up over time in the liver and kidney. Dietary phytate has been shown to increase the level of Cd in the liver and kidney (Rambeck and Walther, 1993; Rimbach et al., 1995). Supplementation of microbial phytase to the diet has been shown to reduce Cd accumulation in rats (Rambeck and Walther, 1993; Rimbach et al., 1995) and Japanese quail (Rambeck and Walther, 1993). However, Rimbach et al. (1996) found that in pigs supplementation of microbial phytase to the diet caused an increase in the concentration of Cd in the liver and kidney. It remains unclear whether the mode of action in pigs is different, or whether the different effects of phytase on Cd accumulation seen in pigs compared to other species is simply due to dietary or study duration differences.

Phytase Effects on Proteins. At a low to neutral pH, the anionic phosphate groups of phytic acid have been shown to possess the ability to bind proteins (Cosgrove, 1980; Prattley et al., 1982; Anderson, 1985; Thompson, 1986) and amino acids; having greatest affinity for the basic amino acids: lysine, arginine, and histidine (Reddy et al., 1982). A typical grow-finish pig diet containing 14% crude protein contains approximately .79 % phytate. Assuming that the average amino acid has a molecular weight of 110, then this diet would contain approximately 12,727 mmoles (1,400,000/110) of amino acids. Likewise, if the amount of phytate in the diet is divided by the molecular weight of phytate this diet contains 12.2 mmoles (7900/648) of phytate. Therefore, for every one amino acid bound by each molecule of phytate, crude protein digestibility decreases by approximately .096% ((12.2/12,727)*100). This value seems quite small, but if each molecule of phytate is binding more than one amino acid or if it is binding di- or tri-peptides, then the value would increase substantially. In addition, phytate may complex with proteases (Singh and Krikorian, 1982) in the gastrointestinal tract, thereby decreasing the activity of these enzymes.

By catalyzing the stepwise hydrolysis of phytate, microbial phytase has the potential to increase protein digestibility by preventing the formation of insoluble phytate-protein complexes in the small intestine. Officer and Batterhan (1992) demonstrated an increased ileal digestibility of crude protein and some amino acids by 7 to 12% when microbial phytase was added to the diet. Mroz et al. (1991) and Khan and Cole (1993) found an increase in ileal crude protein digestibility by 12.8% and 3.5%, respectively. However, Kemme and Jongbloed (1993a,b,c) and Nasi (1990) found no effect of adding microbial phytase on total tract protein digestibility. More recently, Kemme et al. (1995) found an increase in ileal digestibility of amino acids and Jongbloed et al. (1995) and Christensen and Nielson (1995) demonstrated an increase in apparent

total tract digestibility of N. However, Lantzsch and Drochner (1995) showed no improvement in N digestibility when microbial phytase was added to the diets of breeding sows. Addition of phytase has been shown to improve the apparent N absorption in pigs (Kornegay and Qian, 1996; Yi et al., 1996c) and laying hens (Van der Klis and Versteegh, 1991) and the apparent N retention in broilers (Yi et al., 1996d). Yi et al. (1996b) found that adding 750 U/kg of microbial phytase to the diets of turkey poults increased the ileal N and amino acid digestibility and the apparent retention of N.

In a more recent study, Zhang (1999) fed pigs varying levels of CP (10, 11 or 12%) or varying levels of phytase (0, 250, or 500 U/kg) to a low CP diet in an attempt to determine the equivalency values of phytase for CP and amino acids. The response equations which they derived for P and protein/amino acids are shown in Table 11. By setting the phytase and protein/amino equations equal to each other and solving for a set amount of phytase, the phytase equivalency values of CP and amino acids could be derived. Based on the results of this study, 500 U of phytase per kilogram of diet can replace approximately 1.01 percentage units of CP. Estimates of phytase equivalency values for individual amino acids ranged from .03 to .17 percentage units.

Table 11. Estimates of crude protein and amino acid equivalency values of phytase based on the work of Zhang (1999).

	Equations		
Item	Protein	Phytase	Equivalency of 500 U/kg, %
Crude protein (N)	83.13 – 0.6287X, r^2 = 0.91	72.46 + 0.0151X, r^2 = 0.96	1.014
Amino acid CP	83.71 – 0.5011X, r^2 = 0.92	75.17 + 0.0136X, r^2 = 0.99	1.280
Aspartic acid	82.96 – 0.5052X, r^2 = 0.87	73.84 + 0.0175X, r^2 = 0.93	0.148
Threonine	75.82 – 0.6823X, r^2 = 0.94	64.10 + 0.0211X, r^2 = 0.95	0.054
Serine	81.18 – 0.3709X, r^2 = 0.87	74.71 + 0.0138X, r^2 = 0.98	0.074
Glutamic acid	87.69 – 0.4552X, r^2 = 0.98	80.69 + 0.0083X, r^2 = 0.99	0.174
Proline	80.05 – 0.2229X, r^2 = 0.93	76.97 + 0.0056X, r^2 = 0.99	0.103
Glycine	67.42 – 0.3022X, r^2 = 0.18	59.36 + 0.0117X, r^2 = 0.83	0.035
Alanine	82.66 – 0.6524X, r^2 = 0.99	72.23 + 0.0133X, r^2 = 0.90	0.058
Cystine	77.55 – 0.6210X, r^2 = 0.95	68.22 + 0.0167X, r^2 = 0.94	0.030
Valine	82.50 – 0.6947X, r^2 = 0.96	70.61 + 0.0176X, r^2 = 0.88	0.054
Methionine	86.69 – 0.7062X, r^2 = 0.99	75.61 + 0.0142X, r^2 = 0.94	0.018
Isoleucine	84.11 – 0.7258X, r^2 = 0.94	71.43 + 0.0189X, r^2 = 0.88	0.045
Leucine	86.16 – 0.4954X, r^2 = 0.99	78.52 + 0.0081X, r^2 = 0.97	0.083
Tyrosine	81.85 – 0.6241X, r^2 = 0.96	71.53 + 0.0155X, r^2 = 0.88	0.038
Phenylalanine	85.80 – 0.5535X, r^2 = 0.96	76.77 + 0.0134X, r^2 = 0.98	0.059
Histidine	85.87 – 0.4307X, r^2 = 0.95	78.60 + 0.0120X, r^2 = 0.95	0.037
Lysine	84.05 – 0.7640X, r^2 = 0.97	70.84 + 0.0238X, r^2 = 0.89	0.072
Arginine	88.63 – 0.4320X, r^2 = 0.92	81.09 + 0.0126X, r^2 = 0.92	0.082
Meth + Cystine	81.72 – 0.6576X, r^2 = 0.99	71.64 + 0.0155X, r^2 = 0.99	0.047

Antinutritional Effects of β-mannans

Guar gum can be defined as: "a dietary fiber obtained from the endosperm of the seeds of the Indian cluster bean (*Cyamopsis tetragonolobus*)" (Todd et al., 1990). In particular, guar gum consists of galactomannans. Galactomannans consist of a β-(1,4)-linked mannose sugar backbone with β-(1,6)-linked galactose side chains. Interest in guar gum has come from two distinct areas. Initially, it was investigated as a possible protein source for poultry (Nagpal et al., 1971; Verma and McNab, 1982; Patel and McGinnis, 1985). However, the many anti-nutritional effects associated with it have limited its commercial application. More recently, guar gum has been investigated as a dietary method to reduce post-prandiol plasma glucose and insulin levels in individuals suffering from insulin independent diabetes (Rainbird et al., 1984; Sambrook and Rainbird, 1985; Todd et al., 1990). These two uses of guar gum, while at first seeming quite distinct, actually are the result of many of the same digestive characteristics of guar gum.

The anti-nutritional effects associated with guar gum include a decreased gastric emptying, a slower gastrointestinal transit time, a high digesta water holding capacity, and decreased protein retention (Couch et al., 1967; Verma and McNab, 1982; Low and Rainbird, 1984; Rainbird et al., 1984; Patel and McGinnis, 1985; Sambrook and Rainbird, 1985; Brown et al., 1988; Hahn et al., 1995; Anonymous, 1999). These effects stem from two separate anti-nutritional components of guar gum. First, guar gum contains a high level of trypsin inhibitor (Couch et al., 1967), and second, the high galactomannan content. Trypsin inhibitor activity of guar meal can be inactivated by heat processing, but the galactomannan content remains a problem. The anti-nutritional effects associated with galactomannans are extensive, but they all stem from the high water holding capacity of galactomannans.

Patel and McGinnis (1985) investigated the effects of supplementing broiler diets with 10 or 15% guar meal. In broilers, they observed a depression in growth rate and feed efficiency when 15% guar meal was included in the diet. The addition of 10% guar meal did not affect growth rate, but feed efficiency was poorer for birds fed diets containing guar meal. In agreement with this, Verma and McNab (1982), in a large scale floor study (2,000 broilers), observed decreased growth rates over an 8 wk trial when 5, 10, or 15% guar meal was incorporated in the diet. Depressions in growth rate were greater for the first 4 wk, compared to the last 4 wk of the trial. This may indicate a greater tolerance of older broilers to galactomannans, or it may simply reflect a decreased nutrient requirement of older birds. Nagpal et al. (1971) fed guar meal as the sole protein source to chicks. They observed 10 to 30% mortality rates as a result of this diet and noted pancreatic, hepatic, and gall bladder hypertrophy, and spleenic atrophy. These results supported the earlier findings of Couch et al. (1967), who also observed pancreatic hypertrophy when guar meal was included in the diet at a level of 20 or 30%.

Interest in feeding guar meal or guar gum to pigs initially surfaced for the purpose of using pigs as a model for humans to evaluate the effects of a diet high in galactomannans on postprandiol glucose and insulin levels. Rainbird et al. (1984), using four pigs fitted with two re-entrant cannulas placed in the jejunum, found that inclusion of guar gum (6.7 g/L) in a glucose (20 g/L) or maltose (20 g/L) infusion decreased glucose and water absorption from the jejunum. In addition, they found that N secretion was increased (Low and Rainbird, 1984) into the jejunal lumen by guar gum. Later work from the same laboratory (Sambrook and Rainbird, 1985) showed a decrease in the peak post-prandiol glucose and insulin levels in pigs fed diets containing guar meal. More recently, utilizing pigs fitted with gastric cannulas, these researchers

demonstrated that the inclusion of guar gum in the diets of pigs delays gastric emptying, increases gastric pH, and increases digesta viscosity (Rainbird and Low, 1986a; Rainbird and Low, 1986b). Similar results have been observed in the rat, where gastric emptying and cecal filling were both delayed due to the inclusion of guar gum in the diet.

Several studies have demonstrated that the addition of crude or commercial preparations of hemicellulase to the diet decreases some of the detrimental aspects of guar gum when fed to chicks. In 1982, Ray et al., isolated a mannanase from a commercially available hemicellulase preparation. When they added this enzyme to the diet of chicks that contained 2% guar gum, they eliminated the negative impact of the guar gum on growth rate. Interest in a commercially available mannanase preparation grew out of this experiment. However, only recently has it become cost effective to produce a mannanase for use in the feed industry.

Interest in the addition of mannanase to swine diets has been minimal because of the relatively low β-mannan content of most feed ingredients typically used in swine diets. Table 12 shows the estimated β-mannan content of various feedstuffs.

ChemGen Corporation (211 Perry Parkway, Gaithersburg, MD 20877) recently developed a system for producing β-mannanase from cultured *Bacillus lentus* which they now hold a patent for (U. S. Patent No. 5,429,828). The product, known as Hemicell, comes either in liquid or dry form. The liquid product contains 720 million units/L, and the dry product contains 140 million units/kg. Interest in adding this product to swine diets increased following the release of results from a study by the Taiwanese Cugar Company (Anonymous, 1999). They

Table 12. β-mannan content of various feedstuffs.

Feedstuff	β-mannan, % DM
Corn	.09
Soybean meal (48 %)	1.22
Soybean meal (44%)	1.48
Soybean hulls	13
Wheat	.10
Wheat bran	.07
Barley	.49
Oats	.30
Rye	.69
Sorghum	.09
Canola meal	.49
Peanut meal	.51

investigated the addition of .5 kg of Hemicell per ton of feed to grower diets. In that study they compared pigs fed a corn-soybean meal based diet with a low level of barley hull inclusion (.357%) with pigs fed the same diet with the addition of β-mannanase. They observed an increased energy and Ca digestibility for pigs fed the diet supplemented with β-mannanase, compared to pigs fed the unsupplemented diet. Daily fecal excretion was also lower for pigs fed the diet supplemented with β-mannanase.

Methods of Collecting Ileal Digesta

With growing emphasis being placed on ileal digestibility of nutrients, it is essential that swine nutrition researchers have an effective method of collecting digesta at the terminal ileum. Ileal digesta may be collected using a slaughter technique, via an anastamosis procedure or by using a cannulated pig (Sauer and de Lange, 1993; Albin et al., 1999). The slaughter technique is simplistic, but there are many problems associated with it, including only being able to obtain one sample per animal, and problems obtaining representative samples. Anastamosis procedures are not allowed by many animal care committees, and their results are questionable due to the removal of a large section of the gastrointestinal tract. Many cannulation techniques exist including simple T-cannulation, post-valvular T-cecum cannulation, and re-entrant cannulation. All of these techniques allow for collection of ileal digesta, but there are many problems associated with them including non-quantitative collections, collection of non-representative samples, and possible blockages caused by the cannula. Recently, Mroz et al. (1996) implemented a new cannulation technique called steered ileo-cecal valve (SICV) cannulation. This method allows for a quantitative collection of ileal digesta. However, the surgical technique used to insert this cannula is more complex, and has the potential for more post-surgical

complications than the other cannulation techniques. The basic procedures of each of the above techniques along with the advantages and disadvantages of each technique are discussed below.

Slaughter Technique

This procedure involves euthanasia of the pig *via* an approved method followed by removal of the ileum and subsequent collection of digesta. Considerations when using this technique include the method of euthanasia, the length of the terminal small intestine that will be removed, the amount of time after feed consumption that euthanasia occurs, the amount of stress placed on the pig prior to euthanasia, the time it takes to remove the ileum post-mortem, and the completeness of digesta recovery from the removed section (Sauer and de Lange, 1993; Donkoh et al., 1994, Albin et al., 1999).

Approved methods of euthanasia include anesthetic overdose, CO_2 administration, exsanguination (following stunning or under anesthesia), KCl overdose, and electrocution (if justified scientifically). Of those listed, the most commonly used procedures in swine nutrition research are electrical stunning followed by exsanguination, electrocution, and anesthetic overdose (Albin et al., 1999). All of these have some limitations when ileal digesta is being collected. Any use of electrical current may cause cell sloughing in the gastrointestinal tract leading to a contamination of the amino acid content of the digesta (Sauer and de Lange; Albin, 1999). Exsanguination of a stunned animal may also lead to increased cell sloughing. Anesthetic overdosing is preferable when collecting ileal digesta, but care needs to be taken in deciding which anesthetic to use. First, it is essential that the anesthetic chosen does not change smooth muscle contractions and therefore change the rate of digesta passage through the

gastrointestinal tract. Second, the anaesthetic chosen should not alter natural digestive processes by increasing digestive enzyme secretions or by causing a sloughing of enterocytes. Finally, administration method of the anesthetic needs to be considered. Intravenous or gaseous anesthetics may place undue stress on the animal resulting in an increased cell sloughing.

The length of the small intestine removed may affect amino acid digestibility estimates. Di- and tri-peptide transporters are in greatest abundance in the proximal small intestine, but amino acid transporters are in greatest abundance in the distal small intestine (Adibi, 1997). As a result, the longer the section of intestine removed the greater the chance that complete absorption has not occurred. When collecting ileal digesta from the small intestine it is important that the collection comes from the most distal part of the small intestine. The ileum of the pig is generally defined as the area beginning at the proximal attachment of the ileo-cecal band and ending at the ileo-cecal valve. This section is relatively short, usually spanning only 15 to 20 cm (Mroz et al., 1998). Donkoh et al. (1994) found no differences in N digestibility when ileal digesta was removed from the terminal 20 or 40 cm of the small intestine.

The amount of time after meal consumption that digesta is collected will greatly affect amino acid digestiblity calculations since it generally takes about 5 to 6 h efter ingestion for digesta to reach the ileum (Mroz et al., 1993; Donkoh et al., 1994). Even though pigs are often provided *ad libitum* access to feed, they typically consume feed in discrete meals. Therefore, in order to obtain samples of digesta from multiple pigs at the same point in their respective digestive cycles, many researchers have chosen to invoke a fasting and re-feeding regimen or to keep the pigs on a meal fed routine for the duration of the study. Donkoh et al. (1994) investigated the effects of collecting ileal digesta at 3, 4, 5, 6, 7, 9, and 11 h after the start of

feeding. They found no differences in the amount of digesta collected between 5 and 11 h after the start of feeding. However, N digestibility was 9 percentage units higher when digesta was collected at 9 or 11 h after the start of feeding, compared to 5 h. As a result, they concluded that the optimal time to collect ileal digesta was 9 to 11 h after the onset of feeding. It is also important to consider the number of animals per pen when utilizing the slaughter technique. If there is more than one animal per pen, then the start of feeding may differ between animals due to the social hierarchy within pens.

Stress placed on an animal prior to euthanasia can affect the outcome of ileal digestibility analysis (Donkoh et al., 1994; Albin, 1999). As the level of stress increases, the fight or flight response of the animal may be activated. This can result in an evacuation of digesta contents from the small intestine in addition to an increased cell sloughing into the lumen of the intestine.

Ileal digesta needs to be removed from the ileum as quickly as possible following euthanasia to avoid movement of digesta contents and to eliminate contamination of the sample (Donkoh et al., 1994). Removal of digesta needs to be done very carefully. Total recovery of the sample residing in the ileal lumen should be the goal, but over aggressive stripping of the ileum can result in contamination of ileal digesta with amino acid and protein from enterocytes or blood. Washing the ileal lumen with physiological saline may provide the best means of removing all of the digesta from the ileum.

Ileo-rectal anastomosis

The procedure for ileo-rectal anastomosis involves transecting the small intestine just proximal to the ileo-cecal valve and reattaching it to the distal colon (Figure 9; Sauer and deLange, 1993). The cecum and large intestine may remain attached or they may be detached from the colon. The free ends of the large intestine are sewn shut, and generally, the large intestine is left in the abdominal cavity to eliminate the need for redirecting blood supply. Theoretically, by removing the large intestine and cecum, the major microbial populations and their contributions to amino acid breakdown and synthesis in the large intestine are eliminated. This technique is not allowed by animal care organizations in many countries. It has been most heavily used by the French, and it is the basis for the true ileal digestibility estimates published by Eurolysine (Jondreville, 1995). One of the primary concerns with this technique is whether the function of the remaining intact intestine is altered over time due to removal of the large intestine and cecum. Köhler et al. (1992) reported a decreased growth rate, feed conversion, and N retention of ileo-rectal anastomosed pigs compared to normal pigs and pigs fitted with post-valve T-cecal cannulas. Water absorption and energy absorption are also altered (Sauer and de Lange, 1993) by removing the large intestine, and it seems likely that this could affect amino acid digestibilities.

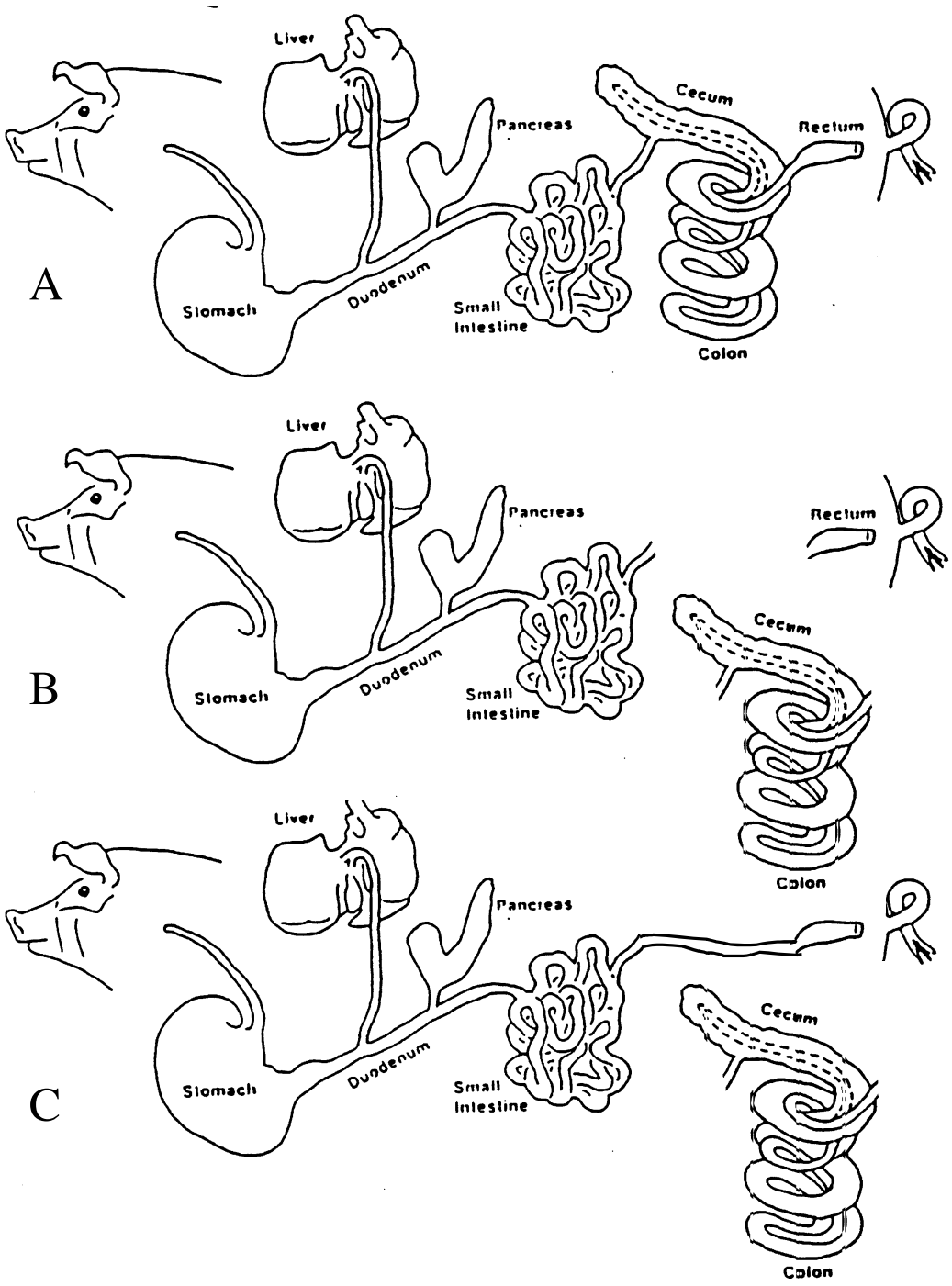


Figure 9. Ileo-rectal anastomosis. A. Normal gastrointestinal tract. B and C. The procedure of ileo-rectal anastomosis involves transection of the distal ileum (B) and ligation of the detached ileum to the terminal colon (C) (Adapted from Moran, 1982).

Simple T-cannulation

This procedure involves insertion of a T-cannula into the terminal ileum. The T-cannula may be a full round T-cannula or a half-round T cannula as shown in Figure 10. The full round T-cannula has the possible advantage of allowing for a more complete collection of ileal digesta. However, some of the liquid phase of the digesta may be able to flow around the cannula. In addition, the full round T-cannula forces digesta to travel through the lumen of the cannula at all times, which can lead to potential blockage problems, especially with high fiber diets. Reduced potential for blockage is the primary advantage of the half-round T-cannula (Sauer and de Lange, 1993; Albin, 1999). Digesta does not travel through the cannula when collections are not occurring. As a result, the chance of the cannula causing a digesta blockage is low. The cannula itself is inserted into the ileal lumen (typically 5 to 10 cm anterior to the ileo-cecal valve) and exteriorized through the body wall, usually on the right side in a region bordered by the last rib, kidney, and the point of the hip (Sauer and de Lange, 1993). This cannulation technique has the advantage of being a relatively quick and simple surgical procedure. Disadvantages include: the inability to quantitatively collect ileal digesta, the possibility that an unequal distribution of the liquid and solid phase digesta may be collected, and the possibility of blocking digesta if a full-round T-cannula is used. In addition, since the cannula is placed in the ileum, the digesta collected has not passed through the entire length of the small intestine..

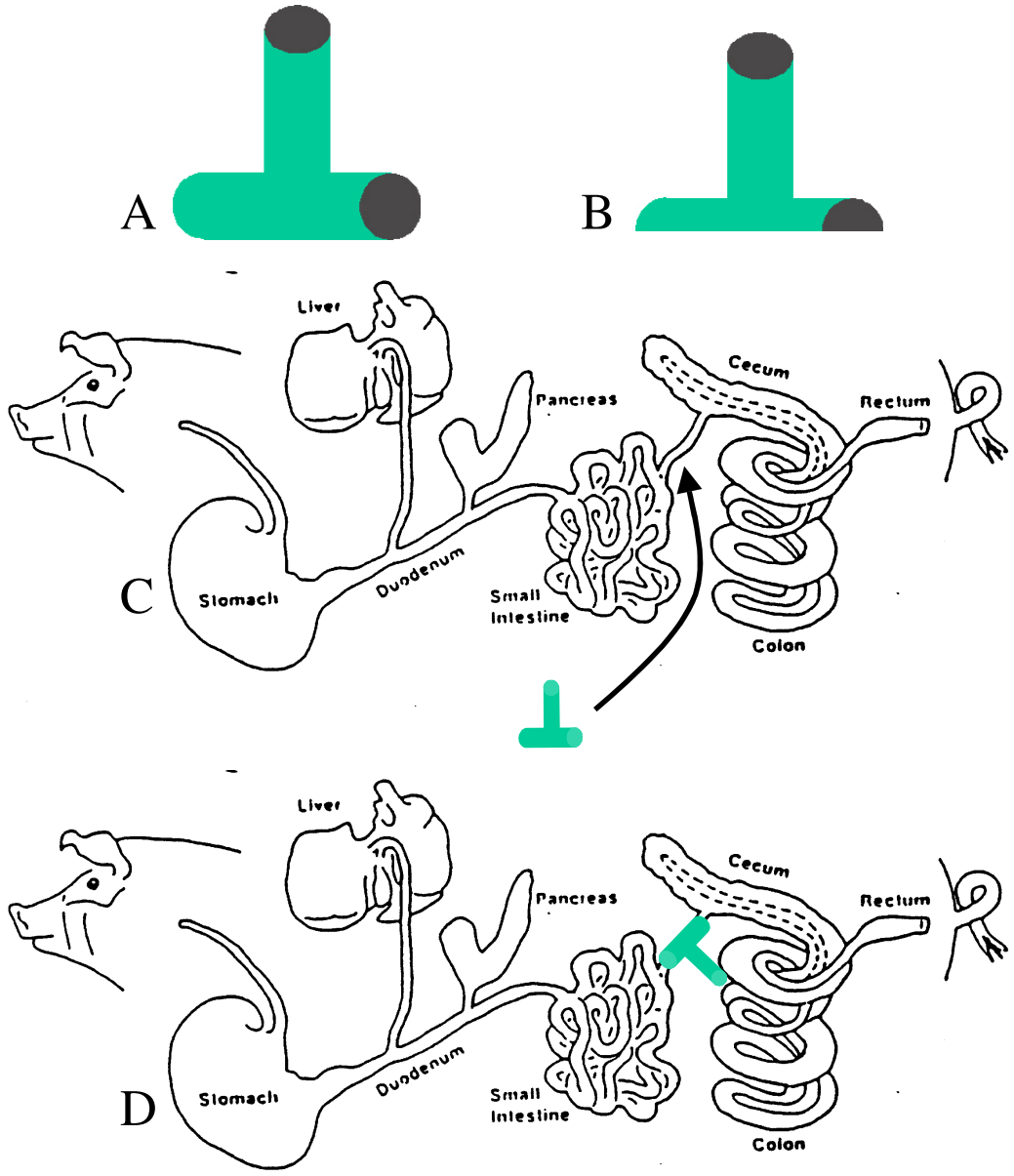


Figure 10. Simple T-cannulation. A. Full-round T-cannula. B. Half-round T-cannula. C and D. The procedure for fitting pigs with simple T-cannulas involves placing a half round or full-round simple T-cannula in the terminal ileum. Typically, the cannula is placed 5 to 20 cm anterior to the ileo-cecal valve (Adapted from Moran, 1982).

Post-valvular T-cecum Cannulation

This technique involves placement of a full round T-cannula at the ileo-cecal junction (Köhler et al., 1992; Sauer and de Lange, 1993; Albin et al., 1999). The cecum is removed, and the T-cannula is placed between ileum and large intestine, allowing digesta to flow from the ileum to the large intestine or out of the pig (Figure 11). This technique has similar advantages and disadvantages to those given above for simple T-cannulation. Additional disadvantages include a more complex surgical procedure and removal of the cecum. Similar to ileo-rectal anastomosis, it remains unclear if the remaining intestine has similar function when another portion of the gastrointestinal tract is removed. However, there are some benefits to this technique compared to simple T-cannulation which include quantitative collection, collection of digesta at a time point when it is normally exited the small intestine, and the use of a larger diameter T-cannula (Sauer and de Lange, 1993; Albin et al., 1999).

Re-entrant Cannulation

Re-entrant cannulation involves diverting the flow of digesta out of the small intestine through a plastic tube and then back into the small intestine via the same tube (Yin et al., 1991; Sauer and de Lange, 1993; Albin et al., 1999). There are several variations of this technique including ileo-ileo, ileo-cecal, and ileo-colic (Sauer and de Lange). All of these techniques involve transecting the small intestine and directing digesta flow outside of the pig through a plastic tube (Figure 12). Digesta, if not collected, is redirected back in the ileum (ileo-ileo), cecum (ileo-cecal) or colon (ileo-colic). During collections, one end of this tube is disconnected and digesta flows out of the pig and into a collection apparatus. Similar to T-cannulation with a

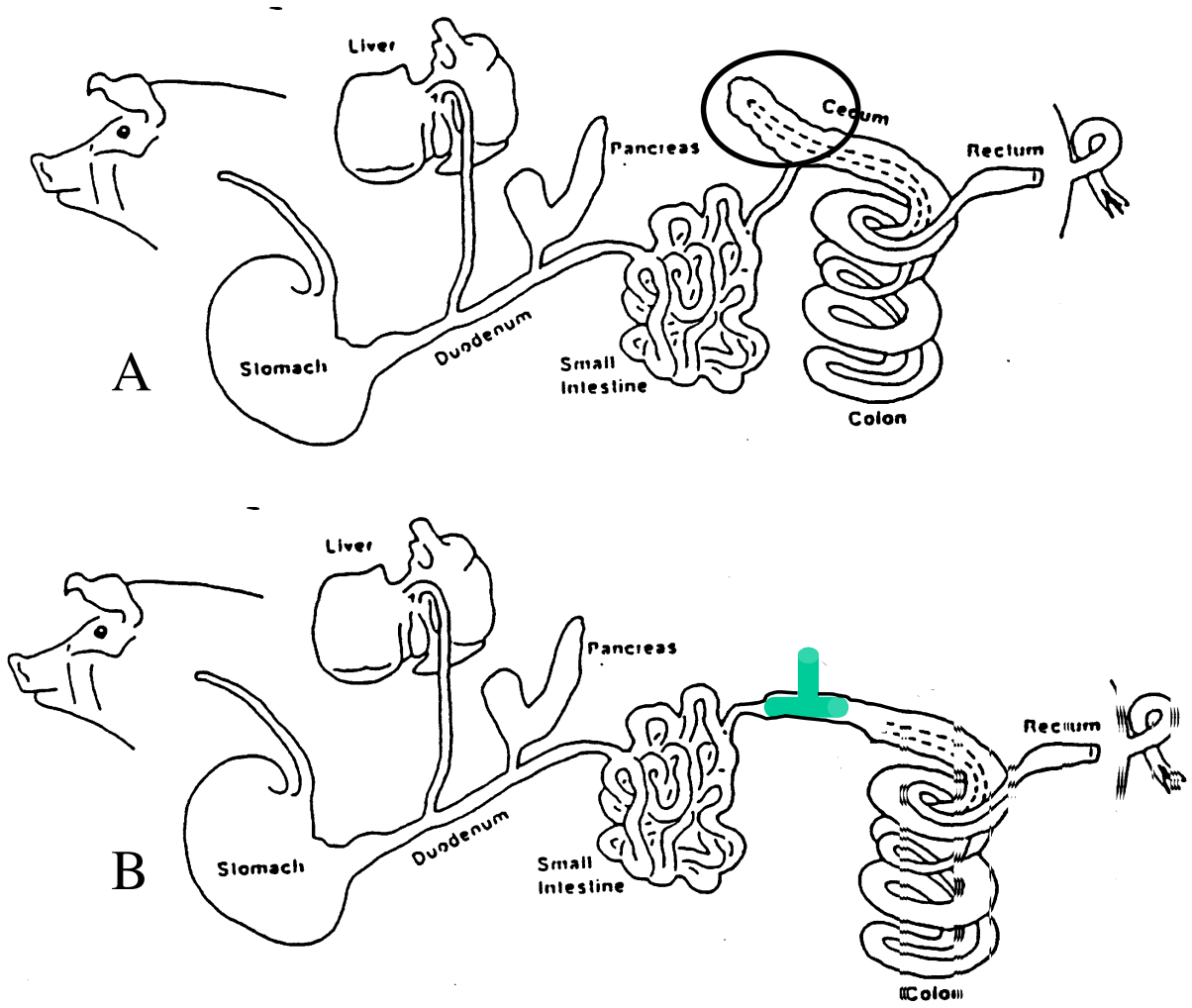


Figure. 11. Post valve T-cecal cannulation. The procedure for fitting pigs with a post valve T-cecal cannula involves removal of the majority of the cecum (A). The remanider of the cecum is used to form a pouch which encloses a simple T-cannula. Therefore, digesta flows from the small intestine, through the cannula barrel, and directly into the large intestine (Adapted from Moran, 1982).

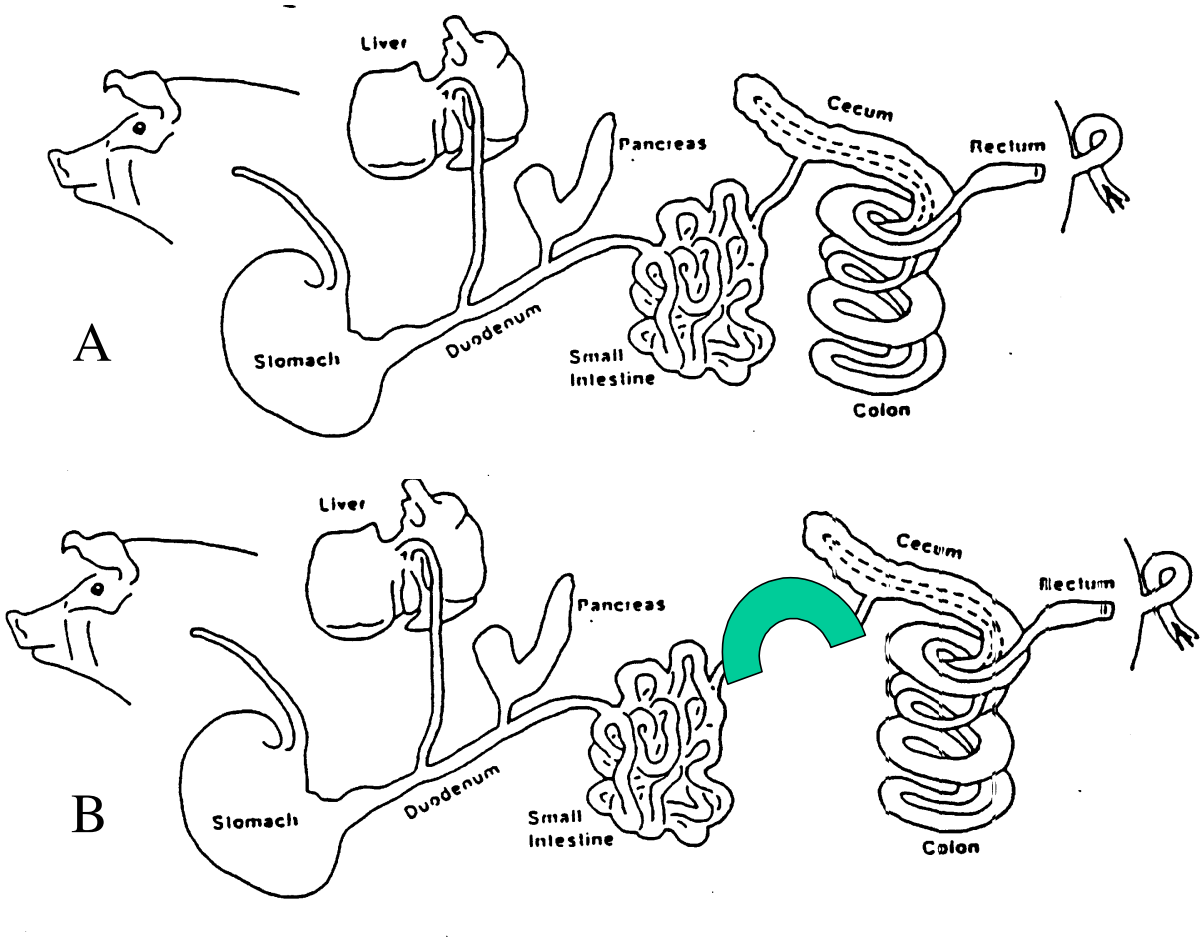


Figure 12. Re-entrant cannulation. The procedure for inserting a re-entrant cannula into a pig involves transection of the ileum and re-direction of the ileum through the cannula and back into the digestive tract. The digesta after passing through the intestine, may be redirected into the ileum (ileo-ileo), cecum (ileo-cecal), or colon (ileo-colic) (Adapted from Moran, 1982).

full-round T-cannula there is a possibility of the cannula causing a blockage because digesta must flow through the cannula lumen at all times. In addition, to divert digesta out of the pig, the small intestine must be severed. Doing this involves cutting through the muscle fibers, and thereby destroying the myoeletic complex that controls peristalsis (Sauer and de Lange, 1993). As a result, the normal processes of digestion may be altered. This technique does, however, allow for a quantitative collection of ileal digesta.

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Chapter III

A Technique for Fitting Pigs with Steered Ileo-Cecal Valve Cannulas

ABSTRACT. Collection of ileal digesta to evaluate amino acid digestibilities has become increasingly important in swine nutrition research. Mroz et al. (1996) developed a method for placing a steered ileo-cecal valve (SICV) cannula into pigs which allows for total collection of ileal digesta and normal flow of digesta during non-collection periods. This technique was modified and used with 46 crossbred barrows in four trials. Procedural changes included incising through the muscle layers in the laparotomy site instead of griding through them, use of a heparinized saline lavage, a stylette in place of a guide ring, and fixing the outer cannula barrel in place with a hose clamp. The current technique involves a right flank laparotomy, parallel and distal to the last rib, with the pigs under general anesthesia. An inner ring (2.0 mm thick, 35.0 mm i.d.) is introduced into the ileal lumen through an enterotomy proximal to the origin of the ileo-cecal band. The string attached to the inner ring is threaded through the ileum and ileo-cecal valve into the cecum using a silastic stylette which encases the string. An outer ring (2.0 mm thick, 34 mm o.d.) is fixed in place around the ileum, distal to the inner ring and just proximal to the ileo-cecal valve. The inner cannula barrel (barrel: 120 mm length, 25 mm i.d., 32 mm o.d.; flange: 70 mm o.d.) is introduced into the cecal lumen *via* an enterotomy through the taenia coli and secured in place with a series of purse string sutures. The inner cannula barrel is exteriorized through a stab wound caudal to the intial laparotomy site where it is plugged using a cylindrical stopper (24 mm o.d., 60 mm length) and held in place by the outer cannula barrel (barrel: 43 mm length, 33 mm i.d., 41 mm o.d.; flange: 80 mm o.d.). These changes led to less post-surgical complications, and less adhesions seen upon necropsy at the end of each experiment. Based on

the four trials, this technique has proven to be a reliable technique for collecting and evaluating ileal digesta.

Key Words: Pigs, Cannula, Ileum, Cecum, Surgery, Amino Acid

Introduction

With growing emphasis being placed on ileal digestibility of nutrients, it is essential that swine nutrition researchers have a reliable method for collecting ileal digesta for digestibility experiments. Ileal digesta may be collected using the slaughter technique, via an anastamosis procedure or by using a cannulated pig. The slaughter technique is simplistic, but there are many problems associated with it, including only being able to obtain one sample per animal, problems obtaining a representative sample, and variation among animals. Anastamosis procedures are not allowed by many animal care committees, and the results are questionable due to removal of a large portion of the gastrointestinal tract. Many cannulation techniques exist, including simple T-cannulation, post-valvular T-cannulation, and re-entrant cannulation. All of these techniques allow for collection of ileal digesta, but there are many problems associated with them, including non-quantitative collections, collection of non-representative samples, and possible blockages caused by the cannula. Recently, Mroz et al. (1996) implemented a new cannulation technique called steered ileo-cecal valve (SICV) cannulation. This method allows for a quantitative collection of ileal digesta. The objectives of this set of experiments were to 1) successfully cannulate pigs with SICV cannulas for use in nutrition experiments, 2) to improve upon the surgical technique for inserting the SICV cannula in order to minimize post-surgical complications, and 3) to improve upon the cannula design to minimize leakage around the cannula.

Materials and Methods

Four experiments were conducted utilizing a total of 50 barrows fitted with steered ileo-cecal valve (SICV) cannulas. In experiment 1, 12 barrows were surgically fitted with SICV cannulas as described by Mroz et al. (1996). In experiments 2, 3, and 4 modifications were made to the procedure in an attempt to minimize post-surgical complications. These modification are discussed in the Results and Discussion section of this chapter and resulted in the development of the following procedure for insertion of SICV cannulas.

Description of Cannula. The SICV cannula consists of 5 parts (Figure 1): an inner cannula barrel (barrel, 100 mm length, 26 mm i.d.; flange, 70 mm o.d.), an outer cannula barrel (barrel, 43 mm length, 33 mm i.d., 41 mm o.d.; flange, 80 mm o.d.), an internal ring (2.0 mm thick and 35.0 mm i.d.) attached to a nylon cord, an external ring (2.0 mm thick and 34 mm o.d.), and a cylindrical stopper (26 mm o.d. and 55 mm length).

Surgical Procedures. Feed was withheld from pigs 36 h prior to surgery and water was taken away 12 h prior to surgery. Anesthesia was induced with an i.m. injection of medetomadine (80 μg/kg BW), ketamine (10 mg/kg BW), and butorphanol (10 mg/kg BW), and pigs were maintained on halothane in oxygen *via* an endotracheal tube for the duration of the surgical procedure. Prior to surgery, pigs were given i.v. injections of Naxcel (Pharmacia and Upjohn Company, Kalamazoo, MI); 2.2 mg/kg BW and Banamine (Schering-Plough Animal Health Corporation, Kenilworth, NJ); 1.1 mg/kg BW.

Pigs were laid on the left side and a laparotomy (70 mm incision) was made at the right hypochondrium. The terminal ileum was located and the inner ring, attached to the nylon cord,

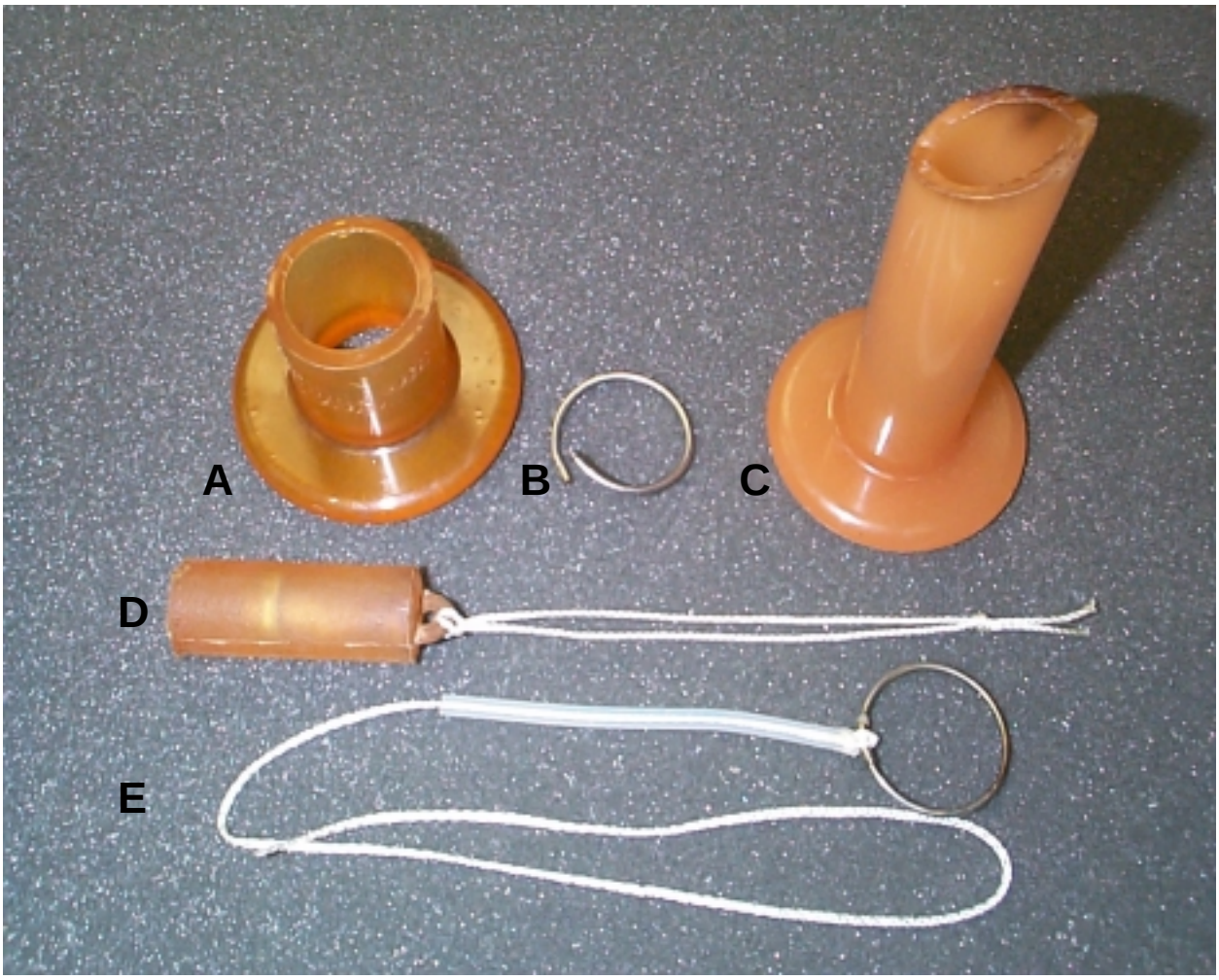

Figure 1. Parts of the steered ileo-cecal valve cannulation system: A) an outer cannula barrel (barrel: 43 mm length, 33 mm i.d., 41 mm o.d.; flange: 80 mm o.d.) B) external ring (2.0 mm thick and 34 mm o.d.) C) inner cannula barrel (barrel: 100 mm length, 26 mm i.d.; flange: 70 mm o.d.) D) cylindrical stopper (26 mm o.d. and 55 mm length) E) internal ring (2.0 mm thick and 35.0 mm inner diameter) attached to a nylon cord.

was introduced into the lumen of the ileum (approximately 15 cm proximal to the ileo-cecal junction) using a silicon stylette (Figure 2). The stylette was passed through the ileo-cecal valve and into the cecum. The incision was closed with 3-0 PDS using a Cushing suture pattern. A purse string suture (approximately 50 mm in length) was placed around the taenia coli of the cecum, and an incision was made in the taenia through which the inner cannula barrel was introduced. The nylon cord attached to the inner ring was threaded through the inner cannula barrel and the purse string suture was drawn tight and secured. A second purse string suture was placed around the cannula for added security. The outer ring of the steering system was secured close to the ileo-cecal junction. The ring was cut so that it could be passed around the ileum and through the ileo-cecal messentary. The ring was connected using a specially designed connecting crimp. The inner cannula barrel was exteriorized through a stab wound (approximately 8 cm distal to the last rib), and the cannula was fixed in place by the outer cannula barrel. A cylindrical plug was placed in the inner barrel to prevent the outflow of digesta. The outer barrel was fixed in placed using a stainless steel hose clamp (21-44 mm diameter) and a plastic wire tie. A four layer closure of the abdominal cavity was performed (transverse abdominus muscle, internal abdominal oblique + external abdominal oblique muscle, subcutaneous tissue, and skin) to close the initial laparotomy site. During surgery a heparanized saline (10 U/mL) lavage was used to keep any exposed tissues moist and to minimize intraabdominal blood clot formation that might lead to post-surgical adhesions.

After surgery, pigs were injected with Banamine (1.1 mg/kg) once daily and Naxcel (2.2 mg/kg) twice daily for 3 d to relieve pain and prevent infection. On the day following surgery

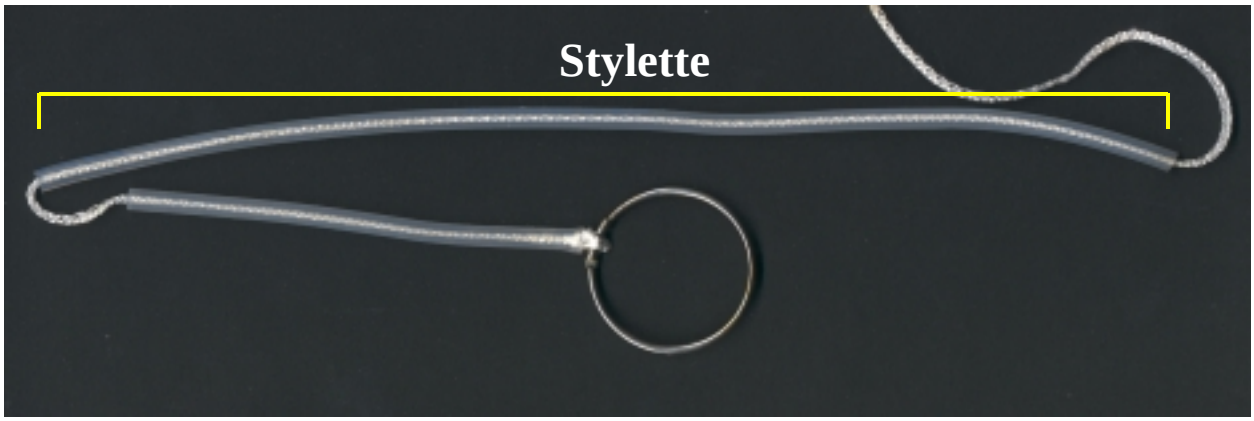

Figure 2. Silicon Stylette

pigs were fed 50 g of feed which was increased 100 g/d up to a level of 9% of their metabolic BW ($BW^{.75}$) All procedures used were approved by the University Animal Care Committee..

Animals, Housing, Experimental Design, and Analysis. Barrows fitted with steered ileo-cecal valve (SICV) cannulas were used in four experiments each consisting of a Latin square type design (Exp. 1: two-5x5 ; Exp. 2: three-4x4; Exp. 3: two-6x6; Exp. 4: two-6x6) to test the effects of added phytase (Exp. 1, 3, and 4) or β-mannanase (Exp. 2) on mineral, amino acid, and energy digestibilities. Pigs were individually housed in specially designed metabolism pens (1.2 m x 1.2 m). Water was supplied *ad libitum* and feed was supplied at a level of 9% of the metabolic BW ($BW^{.75}$) per day. Pigs were fed twice daily (0800 h and 1800 h). Diet types across the four experiments included: corn-soybean meal, corn-soybean meal-wheat midds, corn-soybean meal-meat and bone meal, corn-soybean meal-wheat, corn-wheat-canola, and corn-sorghum-soybean meal based diets. All diets contained .05% chromic oxide as an indigestible marker to allow for calculation of digestion coefficients.

Each 2-wk period consisted of a 7-d adjustment period followed by a 7-d collection period that consisted of a 3-d total collection, a 12-h ileal digesta collection, a 3-d readjustment period and a second 12-h ileal digesta collection (Figure 3). During the 12-h ileal collection, digesta was emptied out of the collection bags and placed on dry ice as soon as it appeared. It was then weighed and placed in an ultra low temperature freezer (-80^{o}C) every hour. Total excretions of feces and urine were collected separately during each 3-d total collection. Feces were collected by placing a plastic bag over the anus of each pig following the procedures of Van Kleef et al. (1994). Urine was collected in buckets from the drop pans under each pen. A 25% HCl solution was added to each bucket to maintain a urine pH below 5. Total urine and fecal

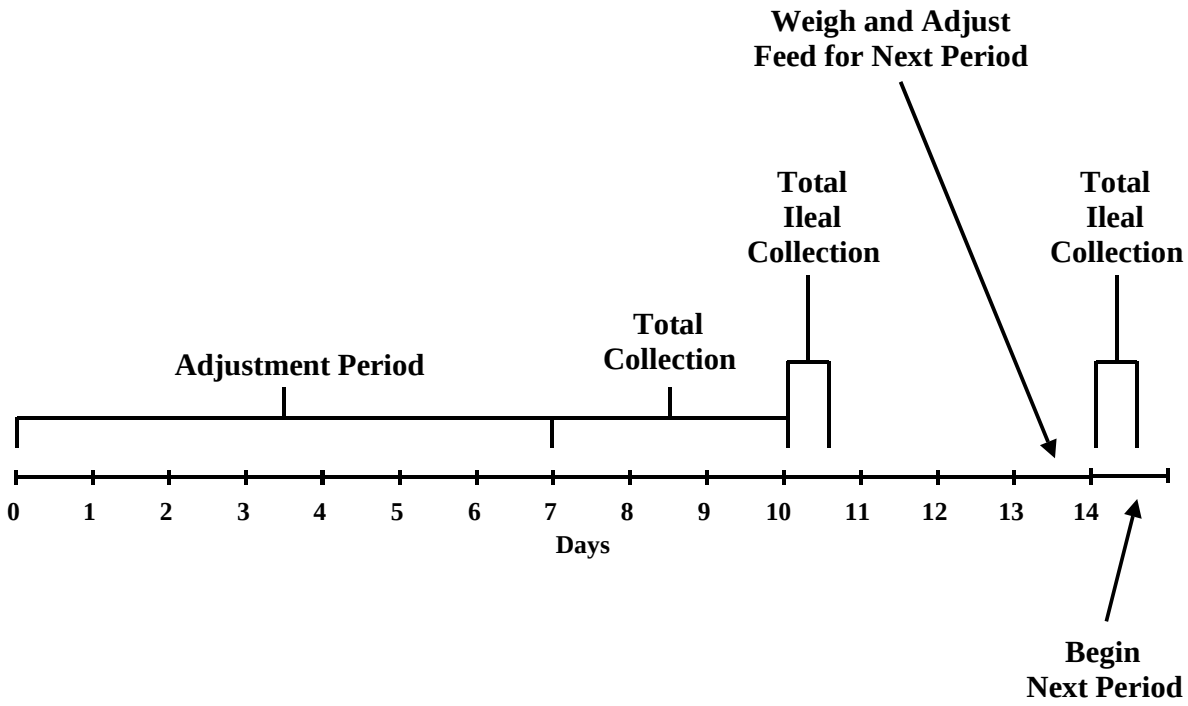

Figure 3. Schedule of each two week period within the Latin square.

samples were collected twice per day during the collection periods and frozen at -20°C for subsequent analysis. Pigs were weighed every 2 wk at the end of the period, at which time feeding levels were adjusted for the subsequent period.

Results and Discussion

These four experiments represent an evolution of the SICV cannulation technique described by Mroz et al. (1996). In experiment 1, surgical procedures were identical to those described by Mroz et al. (1996). Twelve pigs were fitted with SICV cannulas. After a 2 wk adjustment following the last surgery, ten pigs were placed on study and the remaining two were kept as reserves. One of the extra pigs was euthanized 4 wk after surgery because of lack of appetite and a sudden weight loss. Upon necropsy, a jejunal intussusception was observed that was resulting in a blockage of digesta flow. In addition, tissue in the immediate vicinity of the intussusception had become necrotic. The remaining 11 pigs were maintained for the duration of the trial with two of the pigs not consuming all of their feed more than once during the study. At the end of the trial, all pigs were necropsied. The gastrointestinal tract of each pig was classified as being in good, fair, or poor condition based on amount and significance of adhesions involving the distal small intestine. Good condition was defined as no to mild adhesion formation with no impairment of digesta flow. Fair condition was defined as mild to moderate adhesion formation with no impairment of digesta flow. Poor condition was defined as moderate to severe adhesion formation with digesta flow impairment. Five of the eleven pigs were classified as being in good condition, two were classified in fair condition, and three were classified in poor condition. Based on these findings, the surgical procedure described by Mroz et al. (1996) was modified in

an attempt to minimize post-surgical complications. Based on the findings from the necropsies in experiment 1, the distal small intestine appeared to be extremely prone to adhesions. It was thought that adhesion formation might be potentiated by excessive manipulation of the bowel, accumulation of fibrinous exudates and dried blood on the surface of the bowel and within the abdominal cavity, and contamtination (glove powder, bacteria and/or ingesta from the enterotomy site).

Therefore, a two pronged approach was taken to minimize post-surgical complications. In experiments 2, 3, and 4 a heparanized saline lavage (10 U/mL) was used to minimize blood clot formation. In addition, we also regloved immediately following closure of the ileal enterotomy and again following closure of the cecal enterotomy. Gloves were washed with heparanized saline before handling the intestine to remove the glove powder which can act as an irritant and therefore a promotant of adhesions. In experiment 2, two different stylettes were experimented with for threading the string attached to the inner ring through the ileum and ileo-cecal valve into the cecum. One stylette consisted solely of a piece of silastic tubing, while the other was a piece of silastic tubing that encased a small diameter wire to increase its rigidity (Figure 4). The stylette replaced the need for the stainless steel guide ring used by Mroz et al. (1996). By using the stylette, the ileum needed to be handled less, and therefore the potential for post-surgical adhesions was minimized. The string attached to the inner ring was attached to the stylette using a piece of suture (3-0 PDS).

In experiment 2, no feed refusals were observed and all 12 pigs were maintained without incident for the duration of the experiment. At the end of the experiment, all pigs were killed and

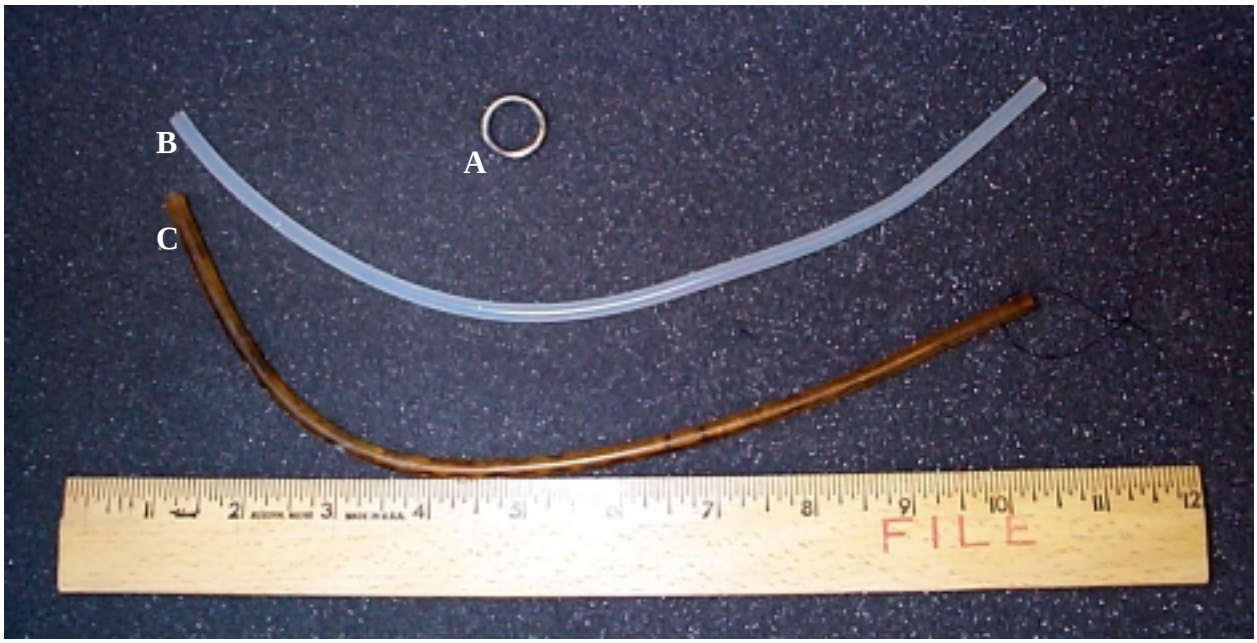

Figure 4. Guide ring and stylettes used to direct the string attached to the inner ring through the ileal lumen and ileo-cecal valve and into the cecum. A) Guide ring as described by Mroz et al. (1996). B) Stylette consisting of a hollow piece of silastic tubing. C) Stylette consisting on a piece of silastic tubing, encasing a thin wire and covered with plastisol.

necropsied to evaluate the surgical technique. Pigs were classified as good, fair, or poor based on the results of the necropsy. Eleven of the 12 pigs were classified as good, and one pig was classified as fair. After this experiment, the stylette was redesigned as a piece of silastic tubing that encased the string attached to the ileal ring. This was done to eliminate the need for feeding the string into the ileum which often led to clumping of the string in the ileal lumen causing unnecessary handling of the ileum to correct.

This newly designed stylette was used in experiment 3 and seemed to aid in insertion and placement of the inner ring.. In addition, to minimize leakage of digesta around the cannula, a stainless steel hose clamp was used to hold the outer cannula barrel in place instead of plastic wire ties. In experiment 3, 14 pigs were surgically fitted with SICV cannulas. Twelve were used in the experiment and two were kept as reserves. All 14 pigs were maintained for the duration of the experiment and no feed refusals were observed. The use of the stainless steel hose clamp to hold the outer cannula barrel in place did appear to decrease the leakage around the cannula. At the end of the experiment, all pigs were killed and necropsied to evaluate the surgical procedure. Twelve of the 14 pigs were classified as being in good condition, and one of the reserve pigs and one of the experimental pigs were classified in fair condition. In this experiment, it appeared that the predominant site for adhesions was the terminal ileum, and that this was due to an inflammatory response caused by the inner ring and the outer ring rubbing against each other and pinching intestine between them. Therefore, in experiment 4, the rings used in the steering system were encased in silastic tubing in an attempt to provide a larger and more forgiving surface area for contact between the two rings (Figure 5).

Figure 5. Outer ring encased in silastic tubing.

In Experiment 4, 12 pigs were fitted with SICV cannulas. Encasing the inner ring with silastic tubing caused the inner ring to slide into and through the ileal lumen less easily. The increased manipulation required to insert the inner ring into the ileal lumen led to contracture of the ileum. Once this contracture had occurred, serosal tearing was observed if the ring was advanced through the ileal lumen. As a result of this observation, the silastic tubing covering the inner ring was removed. However, the silastic tubing was left on the outer ring in all pigs. All 12 pigs were maintained for the duration of the experiment without any feed refusals. At the end of the experiment, all pigs were killed and necropsied to evaluate the surgical procedure. Eight of the pigs were classified in good, and four were classified in fair condition.

Evaluation of amino acid digestibility requires the collection of ileal digesta because fecal amino acid digestibility values are not representative due to microbial activity in the large intestine. Ileal digesta can be collected using a slaughter technique, and anastamosis procedure, or a cannulation technique. The slaughter procedure is limited in use due to problems in obtaining a representative sample, variation among animals, and the small quantity of sample which can be obtained. Anastamosis procedures are not approved for use by many animal care committees due to animal welfare concerns and the results of such trials are questionable due to problems with electrolyte balance in ileo-rectal anastamosed animals. Cannulation procedures include: re-entrant cannulas, simple T-cannulas, post valvular T-cecal cannulas, and SICV cannulas.

Steered ileo-cecal valve cannulas allow for a total collection of ileal digesta, thereby, eliminating the variation of representative sampling techniques. This procedure developed by Mroz et al. (1996) allows for digesta to flow normally when it is not being collected, and it does

not require digesta to pass through the cannula barrel. This eliminates the threat of digesta blockage by the cannulas, particularly when feeding high fiber diets. This technique involves the insertion of a cannula in the cecum and a steering system consisting of two stainless steel rings (Figure 6). One of these rings is located in the ileal lumen and is attached to a string which is threaded through the ileo-cecal vale and out through the cannula barrel, while the other ring is fixed in place around the ileum near the ileo-cecal junction. By pulling on the string attached to the inner ring, the ileo-cecal valve is directed into the lumen of the cannula barrel and digesta can be collected from the pig (Figure 7). This allows for a quantitative collection of ileal digesta. The disadvantage of this technique is the complexity of surgically fitting pigs with the cannula and the subsequent risk of post-surgical complications. This report detailed improvements made to the original procedure described by Mroz et al. (1996) which minimize post-surgical complications. They include: 1) modification of the abdominal approach to allow a larger working field and therefore easier manipulation of the bowel, 2) rinsing gloves with heparanized saline (10 U/mL) prior to entering the abdomen and handling the intestine, 3) use of a heparinized saline lavage to keep the bowel moist and to reduce formation/accumulation of fibrinous exudates, 4) regloving following closure of the ileal enterotomy, 5) use of a styllete for insetion and placement of the internal ring to minimize handling the bowel, and 6) fixing the outer cannula barrel in place with a stainless steel hose clamp to minimize digesta leakage around the cannula. These alterations to the original technique have minimized post-surgical complications, making this a viable research tool for collection of ileal digesta.

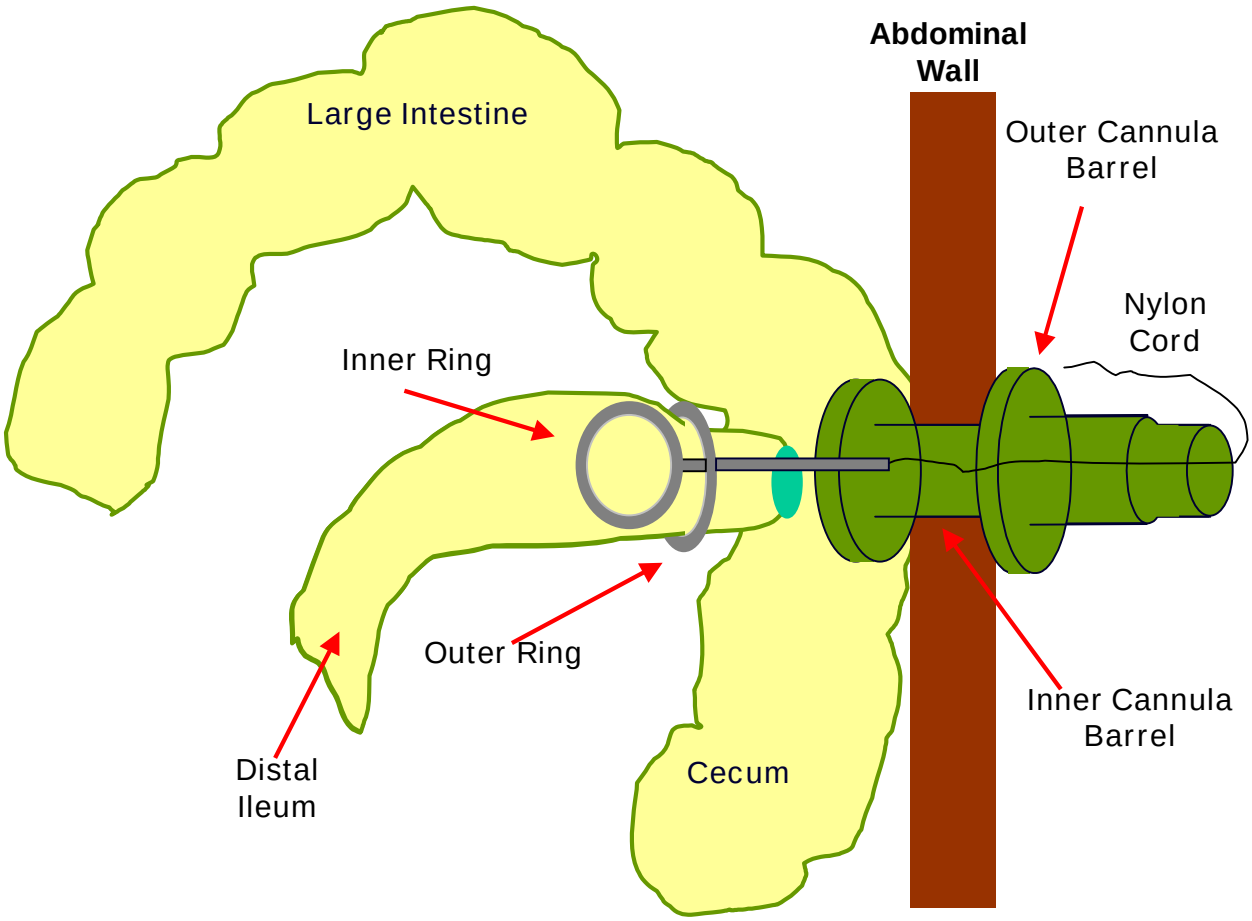

Figure 6. Diagram of the steered ileo-cecal valve cannulation system.

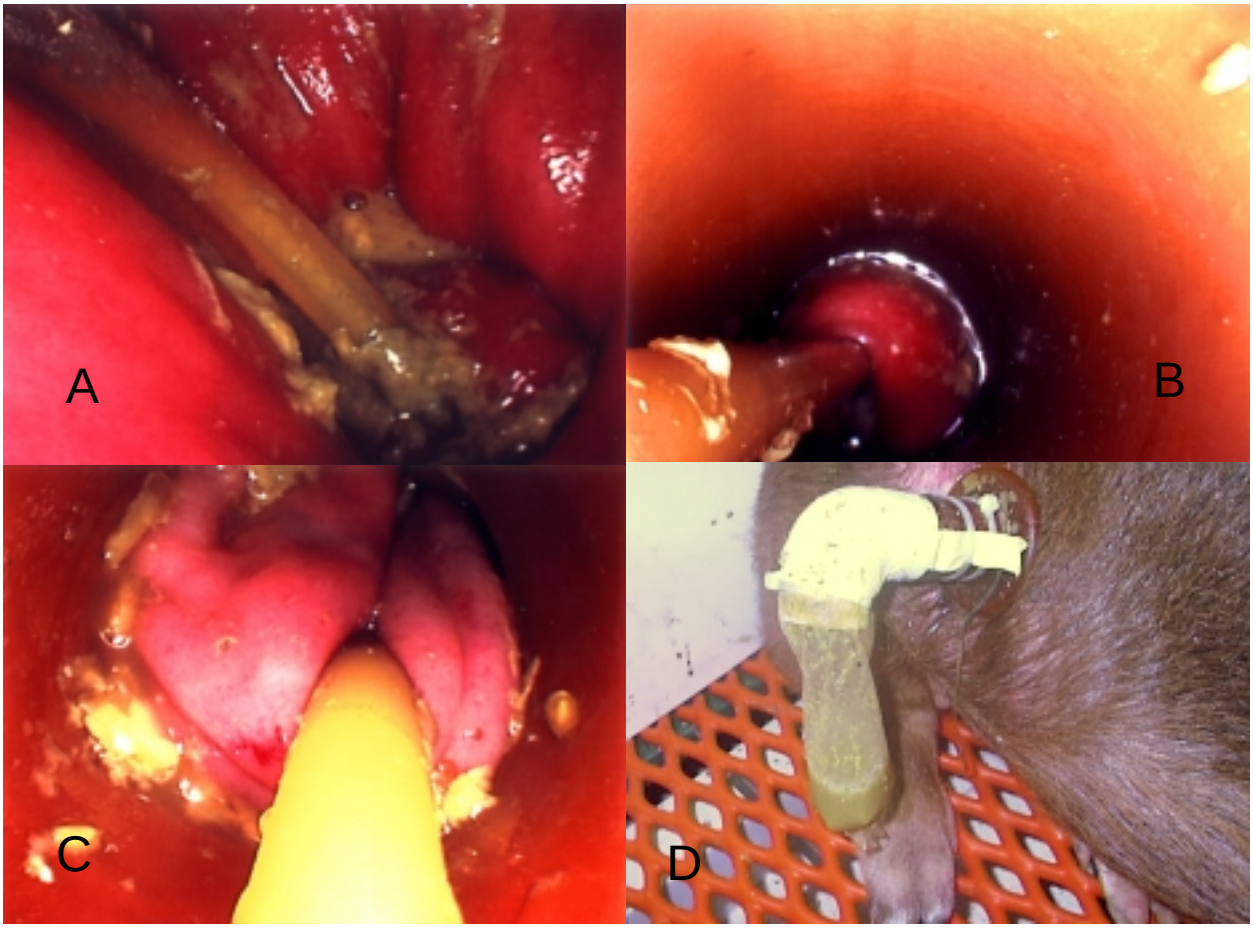

Figure 7. A) Laparoscopic view of the ileo-cecal valve with no tension applied to the string attached to the inner ring. B) Laparscopic view of the ileo-cecal valve as tension is applied to the string and the ileo-cecal valve begins to enter the lumen of the inner cannula barrel. C) Laparoscopic view of the ileo-cecal valve fully inside the inner cannula barrel. D) With tension on the string, the ileo-cecal valve is fully inside the inner cannula barrel and all digesta flows out of the pig through the cannula and can be collected as shown.

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Chapter IV

Estimating Equivalency Values of Microbial Phytase for Amino Acids in Growing and Finishing Pigs Fitted with Steered Ileo-Cecal Valve Cannulas

ABSTRACT. Ten crossbred barrows (48.3 kg initial wt.) fitted with steered ileo-cecal valve (SICV) cannulas were used to investigate the effects of supplemental microbial phytase on the apparent ileal digestibilities (AID) of amino acids, Ca, P, N, and DM, and the apparent total tract digestibilities (ATTD) of Ca, P, N, and DM. All diets were corn-soybean meal-based, and contained .44% Ca and .40% P. Diets 1, 2, and 3 contained 12.0, 11.1, and 10.2% CP, respectively. Diets 4 and 5 had the same ingredient composition as diet 3, plus 250 and 500 U/kg Natuphos® phytase, respectively. Pigs were randomly allotted to one of five dietary treatments in a paired 5 x 5 Latin square with an extra period to test for carry over effects. Each 14-d period consisted of a 7-d adjustment followed by a 3-d total collection, a 12-h ileal digesta collection, a 3-d readjustment, and a second 12-h ileal digesta collection. Pigs were housed individually in metabolism pens (1.2 m x 1.2 m). Water was supplied *ad libitum* and feed given at a level of 9% of the metabolic BW ($BW^{.75}$) per day. Increasing dietary CP increased linearly ($P < .05$) the AID of CP and all amino acids measured with the exception of proline. In addition, the ATTD (g/d) and retention of N (g/d) also increased linearly ($P < .01$) with increasing CP levels. Supplementing diets with phytase increased the AID of Ca ($P < .01$), P ($P < .001$), CP ($P = .07$), and all amino acids ($P < .10$) measured with the exception of leucine, serine, proline, methionine, histidine and tyrosine. Protein and phytase response equations were generated for those amino acids significantly affected by both CP level and phytase supplementation. Based on these equations 500 U/kg of phytase can replace .52 percentage units of the dietary CP which includes

a .03 percentage unit improvement in lysine AID. The results of this study show that supplementing pig diets with microbial phytase improves CP and amino acid digestibilities in addition to Ca and P digestibilities.

Key Words: Phytase, Amino acids, Pigs, Phosphorus, Calcium

Introduction

Sixty to 70% of the P in corn-soybean meal based diets, typically fed to pigs, is bound in phytate. Addition of microbial phytase has been shown to hydrolyze the phytate molecule, releasing the bound P (Nelson et al., 1971; Simons et al., 1990; Cromwell et al., 1993; Kornegay and Qian, 1996) resulting in an increased digestibility of P and a decreased P excretion. The phytate molecule, being extremely anionic, has been shown to bind several cations including Ca , Zn, Cu, and Mn (Maga, 1982; Reddy et al., 1982; Morris, 1986). It has also been shown that the anionic phosphate groups of phytate possess the ability to bind proteins (Prattley et al., 1982) and amino acids (Figure 1), having its greatest affinity for the basic amino acids, lysine, arginine, and histidine (Reddy et al., 1982).

Officer and Batterhan (1992) demonstrated an increased ileal digestibility ofCP and some amino acids by 7 to 12% when microbial phytase was added to the diet. Mroz et al. (1991) and Khan and Cole (1993) also showed an increase in ileal CP digestibility by 12.8% and 3.5%, respectively. However, Nasi (1990) and Kemme and Jongbloed (1993a,b,c) found no effect of adding microbial phytase on total tract protein digestibility. More recently, Kemme et al. (1995) reported an increase in ileal digestibility of amino acids and Jongbloed et al. (1995) and Christensen and Nielson (1995) demonstrated an increase in apparent total tract digestibility of nitrogen. However, Lantzsch and Drochner (1995) showed no improvement in N digestibility when microbial phytase was added to the diets of breeding sows.

The objectives of this study were to evaluate the efficacy of microbial phytase for improving the apparent ileal and/or the apparent total tract digestibilities of amino acids, N, Ca, P, DM, and energy and to calculate equivalency values of microbial phytase for amino acids.

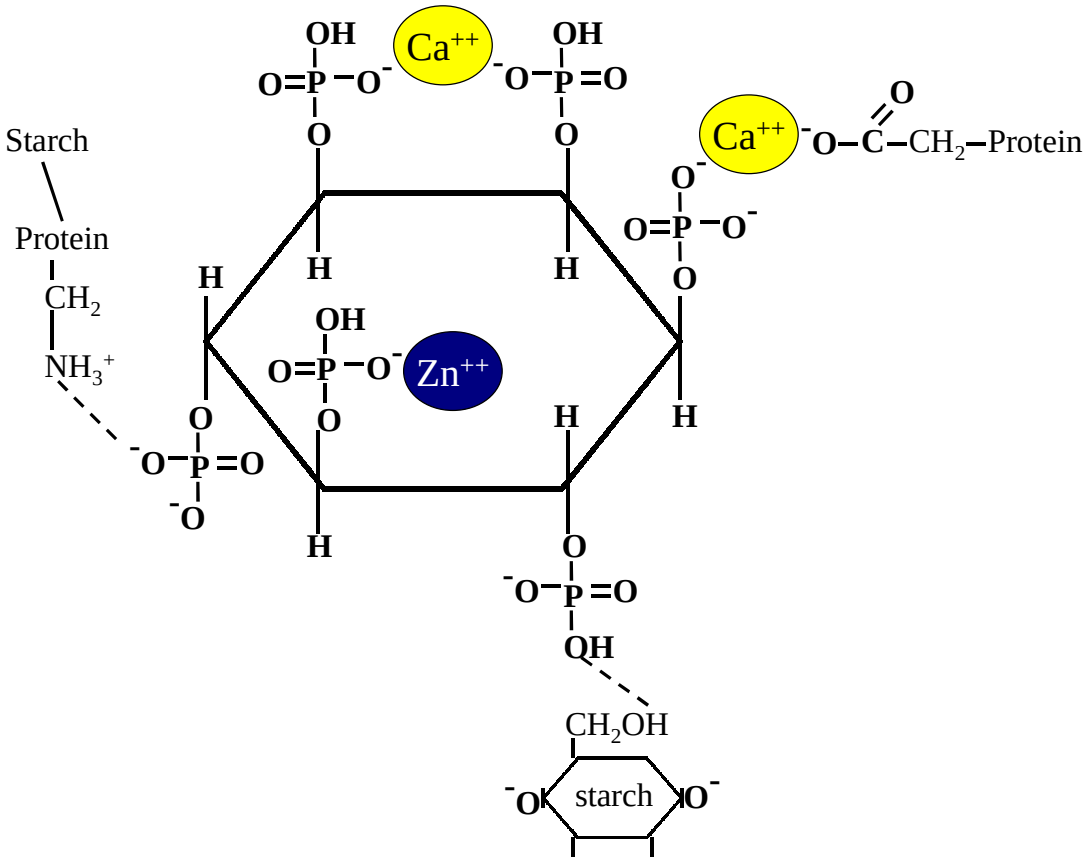

Figure 1. Possible interactions with of minerals, starch and amino acids with the phytate molecule.

Materials and Methods

Animals, Housing, Experimental Design, and Analysis. Ten crossbred barrows fitted with steered ileo-cecal valve (SICV) cannulas (as described in Chapter 3) were used to study the effects of added phytase on mineral, and amino acid digestibilities. Pigs were individually housed in metabolism pens (1.2 m x 1.2 m). Water was supplied *ad libitum* and feed was supplied at a level of 9% of metabolic BW ($BW^{.75}$) per day in two daily feedings (0800 h and 1800 h).

Dietary Treatments. All diets were corn-soybean meal based and formulated to contain adequate levels of all nutrients except crude protein (NRC, 1998). Diets 1, 2, and 3 were formulated to contain 12.0, 11.1, and 10.2 % crude protein, respectively (Table 1). Diets 4 and 5 had the same ingredient composition as diet 3 plus 250 or 500 U of added phytase per kilogram of diet, respectively. Crude protein was lowered in diets 2, 3, 4, and 5 by adding a mixture of corn starch and dextrose (1:1 on a wt:wt basis) to diet 1. The addition of the corn starch-dextrose mixture diluted out the protein level, but allowed the relative proportion of individual amino acids within the diet to remain the same. Chromic oxide was added to all diets as an indigestible marker so that apparent digestion coefficients could be calculated. Pigs were randomly allotted to a paired 5 x 5 Latin square design with one additional period to test for carry over effects (Table 2). Each 2-wk period consisted of a 7-d adjustment followed by a 3-d total collection, a 12-h ileal digesta collection, a 3-d readjustment, and a second 12-h ileal digesta collection (Figure 2). Pigs were weighed every 2-wk prior to the start of the next period at which time feeding levels were adjusted.

During the 12-h ileal collection, digesta was emptied from the collection bags and placed on dry ice as soon as it appeared. It was then weighed and placed in an ultra low temperature

Table 1. Dietary Composition

	Diet				
Item	1	2	3	4	5
Ingredient, %					
Ground corn	87.54	80.25	72.95	72.95	72.95
Soybean meal (44% CP)	10.40	9.53	8.67	8.67	8.67
Limestone	0.82	0.79	0.75	0.75	0.75
Biophos[a]	0.44	0.55	0.67	0.67	0.67
Dextrose	0	4.04	8.08	8.08	8.08
Starch	0	4.04	8.08	8.08	8.08
Minerals and vitamins[b]	0.80	0.80	0.80	0.80	0.80
Phytase, U/kg	0	0	0	250	500
Calculated analysis[c]					
Calcium, %	0.44	0.44	0.44	0.44	0.44
Total phosphorus, %	0.40	0.40	0.40	0.40	0.40
Available phosphorus, %	0.15	0.17	0.19	0.19	0.19
Crude protein, %	12.00	11.10	10.20	10.20	10.20
Total lysine, %	0.52	0.47	0.43	0.43	0.43
ME, kcal/kg	3453	3483	3506	3506	3506
Assayed, %					
Calcium	0.47	0.47	0.47	0.47	0.47
Phosphorus	0.40	0.40	0.40	0.40	0.40

[a]Biophos: a chemical mixture of monocalcium and dicalcium phosphates for poultry and livestock feed (Mallinckrodt Veterinary, Inc. Mundelein Illinois).

[b]Provided 0.30% NaCl and met or exceeded suggested NRC (1998) levels for all other minerals and vitamins.

[c]Calculated values are based on NRC (1998) nutrient levels in corn, soybean meal and dextrose and guaranteed levels in Biophos (21% P and 15% Ca) and ground limestone (Tenn Luttrel Co., Limestone Div., Luttrell, TN, 38% Ca). Available P values are based on NRC (1998) data and assume P bioavailabilities of 15% in corn, 38% in soybean meal and 100% in Biophos.

Table 2. Experimental design.

Block	Pen no.[a]	Period					
		1	**2**	**3**	**4**	**5**	**6**
		Diet					
1	**1**	1	2	3	4	5	5
1	**2**	2	5	4	1	3	3
1	**3**	3	4	2	5	1	1
1	**4**	4	1	5	3	2	2
1	**5**	5	3	1	2	4	4
2	**6**	1	5	2	4	3	3
2	**7**	2	3	5	1	4	4
2	**8**	3	1	4	5	2	2
2	**9**	4	2	1	3	5	5
2	**10**	5	4	3	2	1	1

[a]One pig per pen.

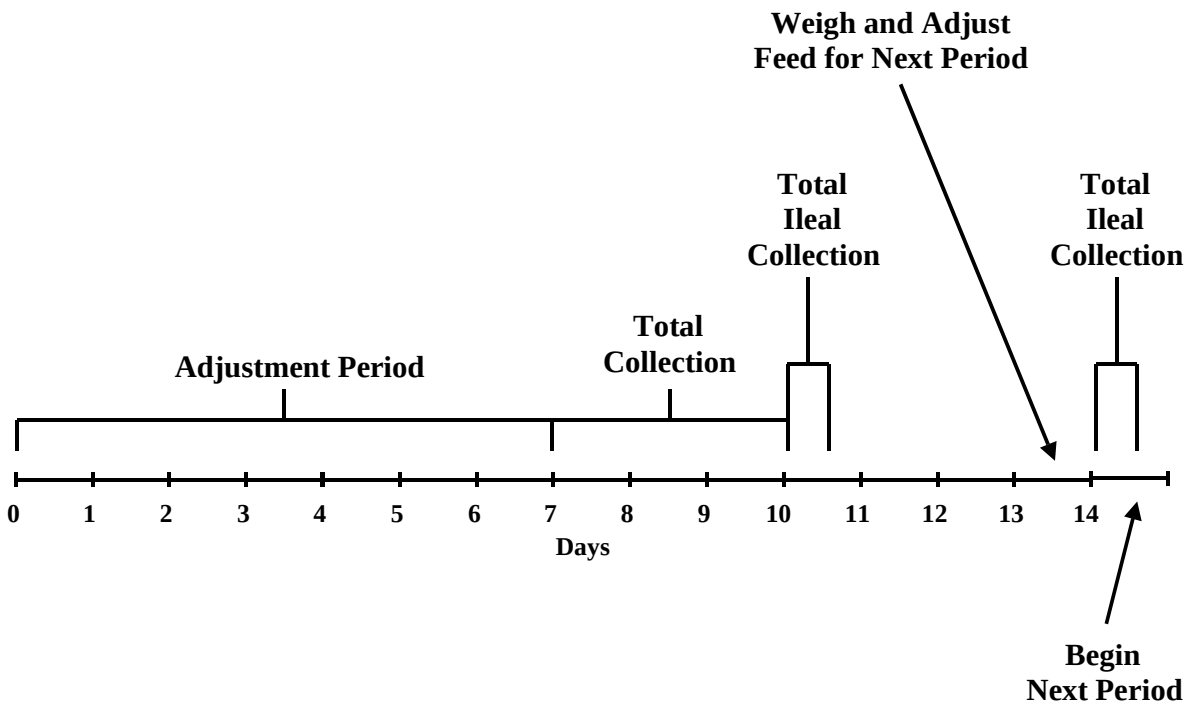

Figure 2. Schedule of each 2-wk period within the Latin square.

freezer (-80^{o}C) every hour. Feces and urine were collected separately during each 3-d total collection. Feces were collected by placing a plastic bag over the anus of each pig following the procedures of Van Kleef et al. (1994). Urine was collected in buckets from drop pans under each pen. A 25% HCl solution was added to each bucket to maintain a urine pH of less than 5. Total urine and fecal samples were collected twice per day during the collection periods and frozen at -20°C for subsequent analysis.

Fecal samples were dried in a forced air oven at 60°C and ileal samples were lyophilized. Dried diet, fecal, and ileal digesta samples were ground to pass through a 1 mm sieve. Diets were analyzed for Ca, P, and Cr following nitric-perchloric acid (5:3, vol/vol) wet digestion. Total P concentrations were assayed photometrically using the vanadomolybdate procedure (AOAC, 1990), and Ca and Cr were determined with an atomic absorption spectrophotometer (model 5100 PC, Perkin elmer, Norwalk, CT) using the manufacturer's recommended procedure. Samples were analyzed for DM content using standard methods (AOAC, 1990). Fecal, urine, and feed samples were digested with sulfuric acid, and N concentrations were assayed using the Kjeldahl procedure (AOAC 1990). Total CP was calculated as N x 6.25. The apparent digestibilities of Ca, P, N, and amino acids were calculated using the indicator method. Diets and ileal digesta were hydrolyzed in 6 N HCl and analyzed for amino acid content using HPLC by the Experiment Station Chemical Laboratories at the University of Missouri-Columbia, Columbia, MO 65211. Gross energy content of the dried feed and feces was determined using an automated bomb calorimeter (Parr, model 1271, Parr Instrument Company, Moline, IL).

Statistical Analysis. Data were analyzed using the GLM procedure of SAS (1990). Pig served as the experimental unit. The model included pen (square), period, treatment, and carry-

over effect. Linear contrasts were used to test the effect of increasing phytase levels or decreasing protein levels and to test the differences between treatment means.

Linear functions were derived for CP levels (diets 1 through 3) and for phytase levels (diets 3 through 5) with the linear model: $Y = a + bX$; where Y = the response measurements; X = 0, 7.5 or 15% CP reduction of the 12% CP diet or 0, 250 or 500 U/kg of phytase added to the reduced CP diet (diet 3). The linear equation for phytase was solved for 500 U/kg of phytase, and the product was set equal to the protein equation and solved. The product was subtracted from 15% (maximum reduction) to give the protein reduction equivalent for 500 U of phytase per kilogram of feed.

Results

Diet Composition. The analyzed dietary compostion was similar to the calculated composition except for phytase activity which was lower than calculated. Crude protein concentrations, as estimated by N concentration in the diet, were 11.8, 11.0, and 10.1 for diets formulated to contain 12.0, 11.1, and 10.2 % CP, respectively (Table 3). Assayed Ca and P levels (Table 1) were also in good agreement with calculated values.

Growth Performance. Pigs grew at an acceptable rate considering that they were limit fed and cannulated (Table 4). There were no effects ($P > .05$) of dietary CP level or phytase supplementation on ADG or feed efficiency, but the effect of phytase approached significance ($P < .08$). This is not surprsing considering the design of the study. Pigs were only on each treatment for 2-wk. One would not expect treatment differences in performance in this time period.

Table 3. Analyzed amino acid and crude protein composition of basal diets used in the finisher phase of the grower-finisher, metabolism, and cannulation studies.

	Diet no.[a]				
Item	1	2	3	4	5
Amino acid, %					
Aspartic acid	1.14	1.08	1.04	1.04	1.04
Threonine	.46	.43	.41	.41	.41
Serine	.53	.51	.49	.49	.49
Glutamic Acid	2.19	2.08	1.99	1.99	1.99
Proline	.84	.79	.75	.75	.75
Glycine	.51	.48	.45	.45	.45
Alanine	.72	.68	.63	.63	.63
Cystine	.26	.24	.22	.22	.22
Valine	.61	.56	.51	.51	.51
Methionine	.22	.20	.19	.19	.19
Isoleucine	.50	.47	.43	.43	.43
Leucine	1.21	1.15	1.07	1.07	1.07
Tyrosine	.38	.36	.35	.35	.35
Phenylalanine	.61	.58	.55	.55	.55
Histidine	.34	.32	.31	.31	.31
Lysine	.60	.57	.54	.54	.54
Arginine	.77	.71	.68	.68	.68
Cystine + methionine	.48	.44	.41	.41	.41
Phytase, U/kg	0	0	0	170	366
Crude protein, %	11.80	11.00	10.10	10.10	10.10
AA protein, %	12.21	11.54	11.10	11.10	11.10
Dry Matter, %	86.33	86.56	86.66	86.66	86.66

[a]Diets 3, 4 and 5 were all created from the same basal diet. The only addition to this basal diet was phytase or corn starch at a level of .083%. Therefore, the average value of amino acids across all three diets is reported.

Table 4. Influence of phytase and protein on growth performance, phosphorus, calcium, and dry matter apparent total tract digestibility, and N balance.

	Diet no.[a,b]							
Item	1	2	3	4	5			
Ca, %	0.44	0.44	0.44	0.44	0.44			
P, %	0.40	0.40	0.40	0.40	0.40			
CP, %	12.0	11.1	10.2	10.2	10.2		Linear effects	
Phytase, U/kg	0	0	0	250	500	MSE	Protein	Phytase
Gain, kg	8.31	8.76	8.16	8.71	6.68	2.28	.8600	.1162
Gain:feed	.289	.307	.292	.312	.235	.076	.8685	.0744
Digestibility, %								
P	49.3	48.5	46.3	53.3	57.6	5.98	.0062	.6681
Ca	64.9	62.8	62.1	65.7	66.4	6.68	.3407	.1224
DM	88.5	89.7	90.6	90.2	90.4	0.90	.2314	.0001
Energy	88.3	88.5	89.5	88.8	89.1	.988	.0043	.3049
N balance								
N intake, g	39.9	37.2	35.7	35.7	36.1	1.88	.0001	.5930
Fecal N, g	5.5	5.7	5.0	5.4	4.9	0.66	.1353	.5890
Urinary N, g	1.6	2.6	2.3	1.6	2.2	1.69	.3799	.8400
N digested, g	34.4	31.6	30.7	30.3	31.2	1.86	.0001	.4640
N retained, g	32.4	28.9	28.4	28.7	29.1	2.84	.0034	.5485
N excreted, g	7.5	8.3	7.3	7.0	7.0	---	---	---
N digested, %	86.3	84.8	85.9	84.9	86.6	1.68	.6459	.3447
N retained, %	81.2	77.6	79.5	80.2	80.4	5.27	.4829	.6726

[a]Treatment mean represents 10 barrows.

[b]SEM = the root mean square error (MSE)/$\sqrt{n}$, where n = 10.

Apparent Total Tract Digestibilities. Calcium and P apparent total tract digestibility (ATTD) were not affected by CP in the diet (Table 4). However, the addition of microbial phytase to the low CP diet linearly increased ($P < .001$) the ATTD of P. Calcium ATTD was numerically improved by the addition of phytase to the diet, but the effect was not significant ($P < .13$). Lowering the level of CP in the diet resulted in a linear increase ($P < .007$) in DM ATTD. Adding phytase to the low CP diet did not affect DM ATTD ($P > .1$).

Nitrogen Balance. Due to the restricted feed intake (9% of $BW^{.75}$), and the fact that there were no feed refusals, daily N intake decreased ($P < .001$) as the level of CP was reduced in the diet (Table 4). Urinary N excretion was similar for pigs fed all diets ($P > .1$). Hence, differences seen in N retention (g/d) were due to changes in fecal N excretion. Nitrogen retention and N ATTD, calculated as a percentage of N intake, were not affected ($P > .1$) by dietary treatment. However, since dietary CP levels were not the same across all diets, it is important to evaluate N retention and ATTD as the amount retained per kilogram of feed intake. When this is done, increasing the level of CP in the diet linearly increased ($P < .004$) the amount of N retained per kilogram of diet by 4.0 g (calculated from Table 4).

Apparent Ileal Digestibility of Ca, P, and DM. Level of dietary CP had no effect on the apparent ileal digestibility (AID) of P or DM (Table 5). Calcium AID tended ($P < .1$) to decrease linearly as the level of CP in the diet was lowered. This response was primarily due to a higher Ca ATTD in the diet containing 12.0% CP, compared to the diets containing 11.1 or 10.2% CP. The addition of microbial phytase to the low CP diet linearly improved the AID of Ca ($P < .004$) and P ($P < .001$) while having no effect ($P > .1$) on DM AID.

Apparent Ileal Digestibility of Amino Acids and Nitrogen. Lowering the level of dietary CP resulted in a linear reduction ($P < .05$) in the AID of all amino acids analyzed except proline

Table 5. Influence of phytase and protein on ileal digestibility of amino acids, dry matter, crude protein (CP), and total amino acid CP of cannulated pigs.

	Diet[a,b]							
Item	1	2	3	4	5			
Ca, %	0.44	0.44	0.44	0.44	0.44			
P, %	0.40	0.40	0.40	0.40	0.40			
CP, %	12.0	11.1	10.2	10.2	10.2		Linear Effects	
Phytase, U/kg	0	0	0	250	500	MSE	Protein	Phytase
Digestibility, %								
P	57.2	53.9	56.5	57.6	66.1	7.3	.7511	.0001
Ca	72.1	67.6	68.7	67.1	74.5	6.4	.0926	.0035
DM	76.2	76.7	77.6	77.0	79.5	4.0	.2579	.1110
CP	73.8	72.0	66.7	69.3	70.1	6.1	.0004	.0704
AACP	80.8	79.7	77.3	78.9	79.9	4.4	.0139	.0566
Nonpolar, aliphatic R groups								
Glycine	69.7	67.9	62.9	67.7	68.7	8.9	.0160	.0315
Alanine	76.1	75.3	71.4	73.1	74.3	5.3	.0062	.0728
Valine	77.4	74.8	70.0	72.5	73.5	5.4	.0001	.0341
Leucine	82.8	81.8	79.9	80.4	81.6	3.6	.0098	.1160
Isoleucine	79.5	77.5	73.0	75.3	75.9	4.7	.0001	.0432
Proline	79.0	80.8	78.9	79.7	81.0	6.9	.9409	.3208
Polar, uncharged R groups								
Serine	81.0	80.2	78.0	78.7	80.4	4.8	.0524	.1116
Threonine	72.3	70.3	66.0	68.2	70.0	6.7	.0038	.0551
Cystine	79.0	77.0	74.7	75.6	77.8	5.2	.0101	.0549
Methionine	79.8	78.6	75.8	77.2	78.0	4.4	.0053	.1188
Met + Cys	79.3	77.7	75.2	76.3	77.9	4.5	.0043	.0579
Aspartic acid	79.6	78.0	74.4	76.8	77.6	5.0	.0014	.0393
Glutamic acid	85.2	84.1	82.3	83.6	84.7	3.9	.0181	.0475

Table 5. (Continued) Influence of phytase and protein on ileal digestibility of amino acids, dry matter, crude protein (CP), and total amino acid CP of cannulated pigs.

	Diet[a,b]							
Item	1	2	3	4	5			
Ca, %	0.44	0.44	0.44	0.44	0.44			
P, %	0.40	0.40	0.40	0.40	0.40			
CP, %	12.0	11.1	10.2	10.2	10.2		Linear Effects	
Phytase, U/kg	0	0	0	250	500	MSE	Protein	Phytase
				Aromatic R groups				
Tyrosine	78.9	77.4	73.8	75.6	76.4	5.2	.0026	.1113
Phenylalanine	82.4	80.9	78.6	79.9	80.8	4.0	.0031	.0732
Histidine	83.9	82.3	80.4	80.7	81.7	3.6	.0025	.2466
				Positively charged R groups				
Lysine	79.4	78.2	72.7	76.3	76.0	5.5	.0002	.0569
Arginine	87.1	85.8	84.0	85.3	85.9	3.1	.0018	.0519

[a]Treatment mean represents 10 barrows.

[b]SEM = the root mean square error (MSE)/$\sqrt{n}$, where n = 10.

(Table 5). On average, amino acid AID was decreased 4.7 percentage units as the CP level of the diet was lowered from 12.0 to 10.2%. Decreases in the AID of amino acids ranged from 2.9 (Leu and Glu) to 7.4 (Val) percentage units. Overall, the AID of CP calculated from N concentration (N x 6.25) decreased ($P < .001$)from 73.8 to 72.0 to 66.7% as the level of dietary CP was decreased from 12.0 to 11.1 to 10.2%, respectively. When phytase was added to the low CP diet, the AID of all amino acids were numerically improved. However, this effect was not significant for Leu ($P < .12$), Pro ($P < .33$), Ser ($P < .12$), Tyr ($P < .12$), His ($P < .25$), and Met ($P < .12$). Apparent ileal digestbility of Gly ($P < .04$), Ala ($P < .08$), Val ($P < .03$), Ile ($P < .05$), Thr ($P < .06$), Cys ($P < .06$), Asp ($P < .04$), Glu ($P < .05$), Phe ($P < .08$), Lys ($P < .06$), and Arg ($P < .06$) were improved an average of 2.9 percentage units when 500 U of phytase were added per kilogram of diet. Improvements in AID ranged from 2.2 (Phe) to 5.8 (Gly) percentage units when phytase was added to the diet. Crude protein AID was tended to be linearly increased ($P =.07$) from 66.7 to 69.3 to 70.1% as the level of added phytase in the diet was increased from 0 to 250 to 500 U/kg, respectively.

Since CP levels differed amongst some of the diets, it is not only important to evaluate amino acid digestibility as a percent of amino acid intake, but also as the amount of each individual amino acid absorbed per kilogram of diet. This was done by taking the digestion coefficient and multiplying it times the concentration of that particular amino acid in the diet. The results of this are shown in Table 6. Decreasing the level of dietary CP resulted in a decreased AID of all amino acids measured ($P < .001$) when reported as the amount of each amino acid digested per kilogram of feed intake. The effects of phytase addition were the same as those reported for AID as a percentage since CP levels were the same in all diets with added phytase.

Table 6. Digested amino acids.

	Diet[a,b]							
Item	1	2	3	4	5			
Ca, %	0.44	0.44	0.44	0.44	0.44			
P, %	0.40	0.40	0.40	0.40	0.40			
CP, %	12.0	11.1	10.2	10.2	10.2		Linear Effects	
Phytase, U/kg	0	0	0	250	500	MSE	Protein	Phytase
Digestibility, g/kg of feed								
			Nonpolar, aliphatic R groups					
Glycine	.419	.373	.331	.350	.354	.0477	.0001	.0359
Alanine	.643	.587	.522	.532	.538	.0400	.0001	.0829
Valine	.555	.481	.14	.425	.431	.0329	.0001	.0395
Leucine	1.171	1.081	.988	.992	1.006	.0460	.0001	.1256
Isoleucine	.467	.48	.364	.373	.375	.024205	.0001	.0486
Proline	.778	.734	.690	.691	.693	.0645	.0001	.3358
			Polar, uncharged R groups					
Serine	.501	.470	.443	.445	.454	.0278	.0001	.1172
Threonine	.392	.346	.315	.322	.330	.0326	.0001	.0598
Cysteine	.241	.212	.190	.192	.197	.01365	.0001	.0621
Methionine	.206	.180	.167	.169	.170	.0102	.0001	.1288
Asparagine	1.064	.968	.898	.920	.928	.0612	.0001	.0420
Glutamine	2.179	2.014	1.897	1.917	1.941	.0906	.0001	.0506
			Aromatic R groups					
Tyrosine	.352	.320	.291	.295	.299	.0210	.0001	.1165
Phenylalanine	.588	.539	.501	.507	.512	.0259	.0001	.0785
			Positively charged R groups					
Lysine	.561	.512	.447	.466	.463	.0346	.0001	.0607
Arginine	.783	.701	.661	.669	.673	.0254	.0001	.0562

[a]Treatment mean represents 10 barrows.

[b]SEM = the root mean square error (MSE)/$\sqrt{n}$, where n = 10.

Equivalency Values. Linear regression was used to develop protein and phytase response equations for amino acid AID as a percent (Table 7) and as the amount digested per kilogram of diet (Table 8). By setting the phytase equation equal to the protein equation and solving for 500 U of added phytase per kilogram of diet, phytase equivalency values for individual amino acids were calculated. The protein equations estimate amino acid digestibility based on a percentage unit reduction in dietary CP content. Diet 1 was formulated to contain 12% CP. Diet 2 was formulated to contain 11.1% CP which represents a 7.5% decrease in CP content relative to diet 1, and diet 3 was formulated to contain 10.2% CP which represents a 15.0% decrease in CP content relative to diet 1. Therefore, the protein response equations describe the response of each amino acid as CP is decreased by 0 to 15%. Therefore, when the phytase response equation is set equal to the protein response equation and solved for a given amount of phytase, the CP value obtained is equal to the decrease in CP. To obtain the percent reduction in CP allowed when phytase is added, this value must be subtracted from 15%. For example, the protein and phytase response equations for aspartic acid are $Y = 80.54 - 0.386X_1$ and $Y = 74.99 + 0.0051X_2$, respectively, where Y = the AID of aspartic acid, X_1 = reduction in CP, and X_2 = added phytase (U/kg). By setting these equations equal to each other and solving for X_1, the following equation is derived: $X_1 = (74.99 + 0.0051X_2 - 80.54)/-0.386$. If this equation is solved for 500 U of phytase activity per kilogram of diet (X_2), then it is determined that $X_1 = 7.77\%$. This means that pigs fed 500 U/kg would perform comparable to pigs fed a diet where the CP had been lowered by 7.77%, or if 7.77% is subtracted from 15.0%, then it is determined that 500 U/kg of phytase can replace 7.23% of the aspartic acid in the diet. To adjust this number to an absolute amount instead of a relative amount, the replacement coefficient (7.23%) was multiplied times the amount of aspartic acid in the diet (1.04 g/kg in the 10.2% CP diet) to arrive at the equivalency

Table 7. Fecal and ileal equations developed for the effects of protein and phytase.

	Protein		Phytase			
Criteria	Equation	r^2	Equation	r^2	Improvement, %[a]	Equivalency[b]
Crude protein (N)	74.99 – 0.5061X	.99	67.48 + 0.0051X	.91	5.20	0.525
Amino acid CP	81.53 – 0.2661X	.99	77.72 + 0.0040X	.99	8.20	0.910
Aspartic acid	80.54 – 0.386X	.99	74.99 + 0.0051X	.93	7.23	0.075
Threonine	73.38 – 0.463X	.99	66.52 + 0.0063X	.99	6.99	0.029
Serine	81.54 – 0.220X	.99	78.11 + 0.0038X	.89	8.05	0.039
Glutamic acid	85.74 – 0.219X	.98	82.57 + 0.0039X	.99	9.43	0.188
Proline	80.18 – 0.0187X	.12	79.66 + 0.00088X	.99	10.72	0.080
Glycine	70.95 – 0.4781X	.99	64.29 + 0.0088X	.87	10.27	0.046
Alanine	77.19 – 0.3513X	.99	71.90 + 0.0043X	.98	6.06	0.038
Cystine	79.61 – 0.3253X	.96	74.72 + 0.0052X	.88	7.96	0.018
Valine	78.49 – 0.5480X	.99	70.53 + 0.0057X	.94	5.68	0.029
Methionine	80.75 – 0.3153X	.99	76.27 + 0.0029X	.94	5.39	0.010
Isoleucine	80.66 – 0.4903X	.99	73.53 + 0.0048X	.89	5.35	0.023
Leucine	83.41 – 0.2324X	.98	79.93 + 0.0028X	.91	6.05	0.065
Tyrosine	79.91 – 0.3829X	.99	74.30 + 0.0041X	.99	5.70	0.020
Phenylalanine	83.00 – 0.2866X	.98	78.91 + 0.0036X	.99	7.01	0.039
Histidine	84.51 – 0.2696X	.96	80.58 + 0.0016X	.78	3.39	0.011
Lysine	81.02 – 0.5093X	.99	73.72 + 0.0051X	.60	5.67	0.031
Arginine	87.65 – 0.2384X	.98	84.32 + 0.0030X	.96	7.32	0.050
Meth + Cystine	80.13 – 0.3203X	.97	75.44 + 0.0041X	.97	6.73	0.028

[a]Based on solving the phytase equations for 500 U/kg of phytase. Then the product is set equal to the protein equations, and the product is substracted from 15% reduction since phytase was added to the 15% reduction diet.
[b]Equivalency = the amount (percentage unit) of CP which can be replaced by 500 U of phytase per kilogram of diet.

Table 8. Amino acid digestibility (g digested per kg feed intake) response equations generated for the addition of CP or phytase to the diet.

	Protein		Phytase			
Criteria	Equation	r^2	Equation	r^2	Improvement, %[a]	Equivalency
Crude protein (N)	1.6505-.0255X	.98	1.2584+.000095X	.91	1.49	.150
Amino acid CP	11.489-.1067X	.98	9.955+.00051X	.98	3.01	.334
Aspartic acid	1.0598-.0111X	.99	.9000+.00006X	.92	3.31	.034
Threonine	.3892-.0051X	.98	.31470+.00003X	.99	3.33	.014
Serine	.5003-.0039X	.99	.4417+.00002X	.89	2.54	.012
Glutamic acid	2.1707-.0188X	.99	1.8962+.000089X	.99	2.77	.055
Proline	.7782-.0059X	.99	.6894+.0000076X	.98	.59	.004
Glycine	.4186-.0059X	.99	.3338+.000046X	.87	4.53	.020
Alanine	.6446-.0081X	.99	.5227+.000031X	.98	1.86	.012
Cystine	.2396-.0034X	.99	.1897+.000013X	.88	2.24	.005
Valine	.5537-.0094X	.99	.4151+.000034X	.94	2.06	.011
Methionine	.2041-.0026X	.96	.1672+.000006X	.93	1.96	.004
Isoleucine	.4680-.0069X	.99	.3649+.000024X	.88	1.80	.008
Leucine	1.172-.0122X	.99	.9869+.000035X	.90	1.26	.014
Tyrosine	.3514-.0041X	.99	.2915+.000016X	.99	2.34	.008
Phenylalanine	.5862-.0058X	.99	.5008+.000023X	.99	2.26	.012
Histidine	.3311-.0030X	.95	.2867-.000013X	.47	-1.97	-.006
Lysine	.5680-.0076X	.99	.4509+.000031X	.60	1.63	.009
Arginine	.7764-.0082X	.96	.6616+.000023X	.96	2.40	.016
Meth + Cystine	.2218-.0030X	.98	.1785+.0000098X	.97	2.20	.009

[a]Based on solving the phytase equations for 500 U/kg of phytase. Then the product is set equal to the protein equations, and the product is substracted from 15% reduction since phytase was added to the 15% reduction diet.

value of .075 g/kg for 500 U/kg of phytase. Based on these calculations, 500 U of phytase per kilogram of diet was capable of replacing 3.39 to 10.75% of the amino acids in the diet. Converting these to absolute equivalency values, 500 U of phytase per kilogram of diet can replace .011 to .188 percentage units of individual amino acids. The effects of phytase on CP digestibility as a whole were evaluated based on N concentration (N x 6.25) or based on the sum of all amino acids in the diet. When estimating CP based on N concentration, 500 U of phytase per kilogram of diet was capable of replacing 5.20% of the CP or .525 percentage units of CP. When estimating total protein using the sum of all amino acids in the diet, 500 U of phytase per kilogram of diet could replace 8.20% of the total protein or .910 percentage units of protein.

Basing the protein response curves for amino acids on AID digestibility as a percent may be misrepresentative of protein utilizatiion by the animal, since the diets used to generate the protein response curves had varying levels (10.2, 11.1, and 12.0%) of CP. Therefore, developing response curves for phytase and protein based on the amount of each amino acid digested per kilogram of diet may provide a more accurate depiction of the AID responses to decreasing dietary CP levels and to the addition of microbial phytase. The results of the regression analysis using digested amino acids (g/kg of diet) are shown in Table 8. By setting these protein and phytase response equations equal to one another and solving for 500 U of phytase per kilogram of diet, it was determined that 500 U of phytase per kilogram of diet can replace from -1.97 to 4.53% of individual amino acids in the diet. Converting this to an absolute amount, it was determined that 500 U/kg of phytase was equivalent to -.006 to .055 percentage units of individual amino acids. It should be noted that His was the only amino acid which provided a negative response, and that His AID, as measured by the amount digested per kilogram of diet, was not significantly affected by the addition of phytase. In addition, linear regression of phytase

level against His AID provided a relatively poor fit (r^2 = .47). Based on solving the response equations of phytase and protein for CP AID as estimated by N concentration (N x 6.25) it was determined that 500 U of phytase per kilogram of diet could replace 1.49% of the dietary CP or .150 percentage units. When the phytase equivalency value of total dietary protein estimated as the sum of all amino acids was determined, it was found that 500 U of phytase per kilogram of diet could replace 3.01% of the total dietary protein or .334 percentage units.

Discussion

Approximately 60 to 80% of the P in plant ingredients typically used in pig diets is bound as phytate P (Cromwell, 1992; Ravindran et al., 1994) which is unavailable to the pig. The addition of microbial phytase to pig diets hydrolyzes some of this phytate P, making it available for absorption by the pig, thus increasing P digestibility (Simons et al., 1990; Jongbloed et al., 1992; Radcliffe and Kornegay, 1998). Kornegay et al. (1998) estimated that 500 U of phytase fed per kilogram of diet was capable of replacing .98 g of P from inorganic P. In the current study, the AID of P was increased from 56.5 to 66.1%, and the ATTD of P was increased from 46.3 to 57.6% when microbial phytase was added to the diet. This 11.3 percentage unit increase in P ATTD represents an increase of .45 g of P digested per kilogram of diet. By dividing .45 g of P by the estimate of P bioavailability from inorganic P derived by Korngay et al. (1998) of 76.7%, it can be concluded that based on the results of this study, 500 U of phytase per kilogram of diet can replace .59 g of P from inorganic P. This value is lower than many reported in the literature (Jongbloed et al., 1992; Korngeay et al., 1998; Radcliffe and Kornegay, 1998), where it has been reported that 500 U of phytase fed per kilogram of diet can replace approximately 1 g of

P from inorganic P. These differences can be explained by the fact that the pigs in the current study were growing and finishing animals in which the P requirement is lower. In addition, some inorganic P was included in the basal diet, and animals were limit fed. All of these factors contribute to a decreased equivalency value estimate due to their effects on decreasing phytase efficacy, increasing the efficiecny of P digestion, and a lowered P requirement of older, heavier weight animals.

In addition to the effect of phytase on P digestibility, phytase has been shown to improve Ca (Radcliffe, 1997) and amino acid digestibilities (Officer and Batterhan, 1992; Khan and Cole, 1993; Jongbloed et al., 1995; Kemme et al., 1995; Zhang and Kornegay, 1999). The mechanism through which phytase causes an increase in Ca and amino acid digestibilities is related to the negative charges carried on each phosphate group of phytate. At a low to neutral pH, the anionic phosphate groups of phytic acid possess the ability to bind amino acids or proteins (Cosgrove, 1980; Prattley et al., 1982; Anderson. 1985; Thompson, 1986), having the greatest affinity for the basic amino acids, lysine, arginine and histidine (Reddy et al., 1982). In this study, the low protein basal diet was formulated to contain 10.2% CP and .78% phytate. If it is assumed that the average amino acid has a molecular weight of 110, then this diet contains 9,091 mmoles (1,000,000/110) of amino acids. Likewise, if the amount of phytate in the diet is dividied by the molecular weight of phytate, then this diet contains 12.0 mmoles (7800/648) of phytate. Therefore, for every individual amino acid bound by each molecule of phytate, crude protein digestibility should decrease by approximately .13% (12.0/9091*100).

The addition of 500 U of phytase per kilogram of diet to the low CP diet resulted in an increase in CP AID from 66.7 to 70.1%. This represents a 3.4 percentage unit increase or a 5.1% increase in CP digestibility. By multiplying 5.1% times the amount of CP in the diet (10.2%) it

is determined that 500 U of phytase per kilogram of diet can replace .52 percentage units of CP. Estimating the phytase equivalency value for CP in this manner assumes that dietary CP is 100% available, which is not the case. Therefore, in this study, multiple levels of CP (10.2, 11.1, and 12.0) and multiple levels of phytase (0, 250, and 500 U/kg) were fed so that response equations for CP and phytase could be developed. Equivalency values were then derived by setting the protein response equation equal to the phytase response equation and solving for a set amount of phytase. Based on protein and phytase response equations for CP AID (%), 500 U of phytase per kilogram of diet can replace 5.20% of the crude protein in the diet or .530 percentage units of CP. This value is in good agreement with those previously reported (Officer and Batterhan, 1992; Khan and Cole, 1993; Mroz et al., 1994; Kemme et al., 1997; Zhang and Kornegay, 1999). Officer and Batterhan (1992) fed semisynthetic diets to pigs weighing approximately 40 kg BW and observed a 12 percentage unit increase in CP AID when 1,000 U of phytase were added per kilogram of diet. By multiplying this increase times the amount of CP in the diet (15.0%) 1,000 U of phytase per kilogram of feed released 1.8 percentage units of dietary protein. Mroz et al. (1994), feeding a 17.0% CP diet to pigs from 45 to 110 kg found a more moderate increase in CP AID of 2.5 percentage units or 3.5%. This translates to 800 U of phytase per kilogram of diet being equivalent to .595 percentage units of dietary protein.

In more recent work, Kemme et al. (1997) reported a 1.6 percentage unit increase or a 2.2% increase in CP AID when 900 U of phytase were added per kilogram of diet to a 13% CP diet fed to pigs from 37 to 95 kg BW. This equates to 900 U of phytase being equivalent to .29 percentage units of dietary protein. Zhang and Kornegay (1999) fed diets identical to those used in this study in a grower-finisher trial and a metabolism trial. They estimated that 500 U of phytase added per kilogram of diet to a 10% CP diet could replace 1.01 percentage units of CP.

Variations in phytase equivalency values for crude protein reported in recent reports occur because of differences in the basal CP content of the diet, differences in the level of phytate P and inorganic P in the diet, differences in the Ca:P ratio, and differences in the grain feedstuffs used in the basal diets. In addition, with the exception of the study by Zhang and Kornegay (1999), only one level of phytase and one level of crude protein without added phytase were fed. Therefore, equivalency values can only be estimated for that one level of phytase addition, and the bioavailability of CP from the diet can not be factored into the equation.

When estimating the phytase equivalency value for CP based on resposne equations generated by feeding multiple levels of protein and multiple levels of phytase to a low CP diet, it may be more correct to estimate equivalency values based on protein response curves for the amount of protein digested per kilogram of diet instead of the percent of dietary intake digested.

Until now, no researchers have attempted to do this when estimating the equivalency value of microbial phytase for CP. However, several studies designed to estimate the P equivalency value of phytase have reported more accurate equivalency estimates using digested P (g/kg of intake) rather than P digestibility as a percent of P intake (Jongbloed, 1996; Radcliffe and Kornegay, 1998). In the present study, the estimate of the CP equivalency of 500 U of phytase per kilogram of diet decreased from .525 percentage units to .150 percentage units when the amount of CP digested per kilogram of diet was used instead of CP digestibility as a percent of protein intake. Using the .150 percentage unit increase and assuming that the average amino acid has a MW of 110, approximately 136 (15,000/110) mmoles of amino acids are being released by phytase. Since there are approximately 12 mmoles of phytate per kilogram of diet, an average of 11.3 amino acids are being released from each phytate molecule by phytase (136/12). If the equivalency value of .525 percentage units is used, that was generated from CP digestibility

data as a percent of CP intake, then phytase is releasing an average of 39.8 amino acids (52,500/110/12) per phytate molecule. This number does not seem realistic. In fact it is questionable whether phytate could bind and phytase could release an average of 11.3 amino acids per phytate molecule, particularly considering that maximal phytate P hydrolysis is only about 60% *in vivo* (estimated from Kornegay et al., 1998).

Therefore, additional actions of phytase on amino acid digestibilities need to be considered. In addition, the accuracy of the above predictions needs to be evaluated. First, the limitations of calculating amino acid equivalency values of phytase using AID should be evaluated. Amino acid digestibilities reported in this paper are referred to as apparent ileal digestibilities because they do not account for endogenous amino acid losses. The major problem with not accounting for endogenous amino acid losses is that the protein response curves generated in this study may provide somewhat misleading results. Endogenous protein losses can be characterized as diet independent losses and diet dependent losses. The diet independent endogenous losses will remain constant regardless of diet type and CP level of the diet. As a result, in this study, pigs fed the lower CP diet had a higher proportion of diet independent endogenous amino acid loss in their ileal digesta compared to pigs fed the higher protein diets. As a result, CP and amino acid AID of these pigs appeared lower than for pigs fed the higher CP diets. Some of this observed effect could be real, but a proportion of it is due to the higher concentration of endogenous CP loss collected in the ileal digesta. Therefore, ideally, AID should be adjusted to true ileal digestibilities by accounting for endogenous protein loss. However, estimating endogenous protein loss is very difficult and all of the techniques available have major limitations (Nyachoti et al., 1997). The net effect of adjusting for endogenous amino acid losses would be that the y intercept of the protein response curve would be higher and the

slope would be lessened. The effect of this on estimates of CP and amino acid equivalency values of phytase would depend on the y intercept and slope of the phytase response curve. Estimates could either decrease or increase.

Another possible mode of action through which phytate may negatively impact amino acid and CP digestibility is through binding and inhibition of proteolytic enzymes. This possiblility has been put forth, but very little research has been conducted to test this hypothesis. Mroz et al. (1991) reported an increase in trypsin activity in duodenal digesta when phytase was added to the diet. Deshpande and Cheryan (1984), using an *in vitro* system, found an inhibition of α-amylase activity when phytate was added to the solution. However, it is unclear if the same type effect would be seen when investigating proteolytic enzymes. If phytate were disrupting proteolytic enzyme activity, it would help explain many of the reported equivalency values of phytase for amino acids and protein, which appear to be too large to be caused by the release of amino acids from phytate alone.

Officer and Batterhan (1992) observed a 7 to 12% increase in amino acid digestibilities for several amino acids when 1,000 U of phytase were added per kilogram of diet. However, the only amino acid significantly improved by phytase addition was lysine. Similarly, Mroz et al. (1994) found a beneficial effect of adding phytase on all amino acids except threonine, but only the effect on methionine was significant. Kemme et al. (1997) reported significant improvements in the range of 1 to 2 percentage units for most amino acids measured. More recently, Zhang and Kornegay (1999) reported improvements in amino acid AID ranging from 7.8% to 15.0%. These estimates were based on response curves for protein and phytase using amino acid AID as a percent of amino acid intake. In the current studies, estimates of the improvement in amino acid AID ranged from 3.39% to 10.72%, with an average improvement of 6.94%. However, when

response curves for protein and phytase were based on the amount of each amino acid digested per kilogram of feed intake, improvements in AID were decreased. With the exception of histidine (1.97% decrease) all amino acid AID were improved from .59% to 4.53%, with the average being 2.3%. When improvements in CP digestibility due to the addition of 500 U of phytase per kilogram of diet were estimated as the sum of all amino acid digestibilities, CP AID was improved by 3.01%. This translates into a phytase equivalency value of .334 percentage units of CP for 500 U of phytase per kilogram of diet.

Implications

The utilization of amino acids, P, and Ca by pigs are improved when plant-based diets are supplemented with microbial phytase and dietary P, Ca and CP levels are appropriately reduced. Based on the results of this study, the CP content of the diet can be reduced by approximately .15 percentage units when 500 U of phytase are added per kilogram of diet. Additional research needs to be conducted to account for endogenous protein losses in the small intestine of the pig so that more accurate equivalency values can be developed.

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Chapter V

The effects of microbial phytase on amino acid, mineral, and energy digestibilities in grow-finish pigs fitted with steered ileo-cecal valve cannulas and fed corn-soybean meal, corn-soybean meal-wheat middlings, or corn-soybean meal-meat and bone meal based diets.

ABSTRACT. Twelve crossbred barrows fitted with steered ileo-cecal valve (SICV) cannulas were used in a paired 6 x 6 Latin square design to test the effects of added phytase on amino acid, mineral, and energy digestibililties. Pigs had *ad libitum* access to water, and feed was supplied at 9% of metabolic body weight ($BW^{.75}$) in two daily feedings. Pigs were fed corn-soybean meal (CS), corn-soybean meal-wheat middlings (CSWM), or corn-soybean meal-meat and bone meal (CSMB) based diets with or without 500 U of phytase/kg of diet in a 3 x 2 factorial arrangement. Each 2-wk period consisted of a 7-d adjustment, a 3-d total collection, a 12-h ileal digesta collection, a 3-d readjustment period, and a second 12-h ileal digesta collection. Pigs were individually housed in metabolism pens (1.2m x 1.2m). Upon analysis, diets formulated to contain 500 U of added phytase/kg of diet contained an average of only 319 U/kg of the diet. Wheat contributed an additional 250 U of endogenous phytase activity/kg of diet in the CSWM based diets. The addition of microbial phytase to all diet types increased ADG (P<.05) and tended to increase feed efficiency (P=.056). Phytase addition also increased apparent total tract digestibility (ATTD) and apparent ileal digestibility (AID) of Ca (P<.001) and P (P<.001), but had no effect on amino acid AID. Pigs fed the CSMB based diet had higher (P<.05) ATTD and AID of Ca and P compared to pigs fed the CS or CSWM diets. Energy ATTD was higher (P<.05) for pigs fed the CSMB based diet, compared to pigs fed the CSWM based diet. The AID of Asp, Thr, Ser, Glu, Gly, Ala, Met Tyr, His, and Lys were affected (P<.01) by diet type. However, no diet was consistently superior to any other diet with regards to amino acid AID.

The results of this study demonstrate that phytase improves Ca and P digestibilities equally in CS, CSWM, and CSMB based diets.

Key Words: Pigs, Phytase, Amino acids, Energy, Minerals, Cannula

Introduction

Interest in the addition of microbial phytase to pig diets originally stemmed from the ability of the enzyme to release phytate P, making it available for digestion and absorption. Many research trials have been conducted to quantify the effects of adding phytase to pig diets on P availability. An added finding of many of those trials has been the observation that adding phytase to the diet affects more than just P digestibility when added to the diet. In fact, the addition of microbial phytase to pig diets has been shown to increase the digestibility of Ca (Radcliffe et al., 1995; Jongbloed et al., 1996), Zn (Pallauf et al., 1992; Lei et al., 1993c; Nasi and Helander, 1994), energy (Ketaren et al., 1993), and amino acids (Mroz et al., 1991; Officer and Batterhan, 1992; Khan and Cole, 1993; Christensen and Nielson, 1995; Kemme et al., 1995; Jongbloed et al., 1995; Kornegay and Qian, 1996; Yi et al., 1996c; Zhang and Kornegay, 1999). The effect of phytase on amino acid digestibility is of particular interest for two reasons. First, dietary addition of plant based feed ingredients, high in CP, in order to meet the CP requirement of pigs is expensive. Second, excess N excretion has the potential to lead to increased environmental pollution. In order to adjust diet CP levels appropriately when phytase is added to the diet, equivalency values of phytase for CP are needed. More specifically, amino acid equivalency values of phytase are needed. Interest in determining amino acid equivalency values for phytase has grown in the last several years. However, very few scientists have attempted to determine amino acid equivalency values of phytase. Ileal digesta must be collected to estimate amino acid digestibilities because microorganisms in the large intestine alter the amino acid profile that is excreted. In the majority of studies designed to investigate the effects of phytase on amino acid digestibilities, corn-soybean meal based diets have been fed because these are the

major diet type fed in the United States (Kornegay et al., 1998). However, in other countries, many more plant feed ingredients are utilized in the diet. In addition, increasing demands on U. S. corn and soybean meal may cause the price of these ingredients to rise. If this happens, then U. S. swine producers may be searching for alternative feedstuffs to use in diets. Therefore, this study was designed to investigate the effects of microbial phytase on amino acid digestibilities in corn-soybean meal, corn-wheat middlings-soybean meal, and corn-soybean meal-meat and bone meal based diets.

Materials and Methods

Animals, Housing, Experimental Design, and Analysis. Twelve crossbred barrows (average initial weight = 46 kg) fitted with steered ileo-cecal valve (SICV) cannulas (as described in Chapter III) were used in two 6 x 6 Latin squares (Table 1) to test the effects of added phytase on mineral, amino acid, and energy digestibilities. Pigs were individually housed in metabolism pens (1.2 m x 1.2 m). Water was supplied ad libitum and feed was supplied at a level of 9% of metabolic BW ($BW^{.75}$) per day. Pigs were fed twice daily at 0800 h and 1800 h. Dietary treatments are shown in Table 2 and compositions of the basal diets are shown in Table 3. Diets 1 and 2 were corn-soybean meal based (CS), diets 3 and 4 were corn-soybean meal-wheat middlings (CSWM) based, and diets 5 and 6 were corn-soybean meal-meat and bone meal (CSMB) based. Microbial phytase (Natuphos 600) was added at a level of 500 U/kg to diets 2, 4 and 6. All diets contained .05% chromic oxide as an indigestible marker for calculation of digestion coefficients. Pigs were weighed every 2 wk at the end of each period, at which time feeding levels were adjusted.

Table 1. Experimental design.

	Latin square/pen no.[a]											
	Latin square no. 1						Latin square no. 2					
Period	1	2	3	4	5	6	7	8	9	10	11	12
1	1[b]	2	3	4	5	6	1	2	3	4	5	6
2	6	1	2	3	4	5	6	1	2	3	4	5
3	2	3	4	5	6	1	2	3	4	5	6	1
4	5	6	1	2	3	4	5	6	1	2	3	4
5	3	4	5	6	1	2	3	4	5	6	1	2
6	4	5	6	1	2	3	4	5	6	1	2	3

[a]One pig per pen.
[b]Diet no.

Table 2. Dietary treatments.

	Diet no.					
Item	1	2	3	4	5	6
Basal	CS[a]	CS[a]	CSWM[b]	CSWM[b]	CSMB[c]	CSMB[c]
Corn starch (%)	.083	---------	.083	---------	.083	---------
Phytase (U/kg)	---------	500	---------	500	---------	500

[a]CS = corn-soybean meal based diet.
[b]CSWM = corn-soybean meal-wheat midds based diet.
[c]CSMB = corn-soybean meal-meat and bone meal based diet.

Table 3. Composition of basal diets.

	Diet				
	1&2	3&4	5&6	NRC, 1998 Requirement	
Item	CS[a]	CSWM[b]	CSMB[c]	50-80 kg	80-120 kg
Ingredient composition, %					
Corn	85.24	65.47	87.50		
Wheat Midds	---	20.00	---		
Limestone	0.78	0.82	0.08		
Lysine HCl	0.03	---	0.13		
Fat	---	2.08	0.66		
Meat and bone meal	---	---	6.00		
SBM	12.55	10.31	4.99		
Salt	0.33	0.33	0.21		
Dicalcium phosphate	0.64	0.56	---		
Vit. Premix	0.05	0.05	0.05		
TM premix	0.05	0.05	0.05		
Se premix	0.05	0.05	0.05		
Cr-premix	0.20	0.20	0.20		
Empty space[d]	0.08	0.08	0.08		
Calculated composition					
CP, %	13.00	13.00	13.00	15.5	13.2
Lys, %	0.62	0.64	0.63	.75	.60
Ileal digestible Lys, %	0.49	0.50	0.49	.61	.47
Met, %	0.23	0.23	0.22	.20	.16
Ileal digestible Met, %	0.20	0.20	0.19	.17	.13
Trp, %	0.13	0.15	0.10	.14	.11
Ileal digestible Trp, %	0.10	0.11	0.07	.10	.08
Thr, %	0.48	0.48	0.44	.51	.41
Ileal digestible Thr, %	0.35	0.35	0.31	.37	.30
ME (kcal/kg)	3342	3350	3350	3265	3265
Ca, %	0.50	0.50	0.50	.50	.45
P, %	0.44	0.45	0.50	.45	.40
Available P, %	0.17	0.17	0.25	.19	.15
Na, %	0.15	0.15	0.15	.10	.10
Crude fat, %	3.70	5.54	4.87	---	---
NDF, %	9.30	14.32	10.79	---	---
ADF, %	3.06	4.53	3.06	---	---

[a]CS = corn-soybean meal based diet.
[b]CSWM = corn-soybean meal-wheat midds based diet.
[c]CSMB = corn-soybean meal-meat and bone meal based diet.
[d]Empty space was replaced with corn starch or phytase.

Each 2-wk period consisted of a 7-d adjustment period followed by a 7-d collection period that consisted of a 3-d total collection, a 12-h ileal digesta collection, a 3-d readjustment period and a second 12-h ileal digesta collection. During the 12-h ileal collection, digesta was emptied out of the collection bags and placed on dry ice as soon as it appeared. It was then weighed and placed in an ultra-low temperature freezer (-80^{o}C) every hour. Total excretions of feces and urine were collected separately during each 3-d total collection. Feces were collected by placing a plastic bag over the anus of each pig following the procedures of Van Kleef et al. (1994). Urine was collected in buckets from drop pans under each pen. A 25% HCl solution was added to each bucket to maintain a urine pH of less than 5. Total urine and fecal samples were collected twice per day during the collection periods and frozen at -20°C for subsequent analysis.

Fecal samples were dried in a forced air oven at 60°C and ileal samples were freeze dried. Diet, fecal, and ileal digesta samples were ground to pass through a 1 mm screen and analyzed for Ca, P, and Cr, following nitric-perchloric acid (5:3, vol:vol) wet digestion. Total P concentrations were assayed photometrically using the vanadomolybdate procedure (AOAC, 1990) and Ca and Cr were determined by atomic absorption spectrophotometry following the manufacturer's (model 5100 PC, Perkin Elmer, Norwalk, CT) recommended procedures. Samples were analyzed for N content using the Kjeldahl procedure (AOAC, 1990). Diets and ileal digesta were hydrolyzed in 6 N HCl for 24 h and analyzed for amino acid content using HPLC by the Experiment Station Chemical Laboratories at the University of Missouri-Columbia (Columbia, MO 65211). A bomb calorimeter (Parr Model 1271, Parr Instrument Company, Moline, Il) was used to determine gross energy of feed, ileal and fecal samples.

Description of Cannula. The cannula was described in Chapter III. Briefly, the SICV cannula consists of 5 parts (Figure 1): an inner cannula barrel (barrel, 100 mm length, 26 mm i.d.; flange, 70 mm o.d.), an outer cannula barrel (barrel, 43 mm length, 33 mm i.d., 41 mm o.d.; flange, 80 mm o.d.), an internal ring (2.0 mm thick and 35.0 mm inner diameter) attached to a nylon cord, an external ring (2.0 mm thick and 34 mm o.d.), and a cylindrical stopper (26 mm o.d. and 55 mm length).

Surgical Procedures. Surgical procedures for the insertion of the SICV cannula were as described in Chapter 3. Breifly, pigs were fasted from feed for 36 h prior to surgery and water was taken away 12 h prior to surgery. Anesthesia was induced with an i.m. injection of medetomadine (80 μg/kg BW), ketamine (10 mg/kg BW), and butorphanol (10 mg/kg BW), and pigs were maintained on halothane in oxygen *via* an endotracheal tube for the duration of the surgical procedure. Prior to surgery pigs were given an I.V. injection of Naxcel® (1 mg/lb BW) and banamine (.5 mg/lb BW). On the day following surgery pigs were fed 50 g of feed which was increased 100 g/d up to a level of 9% of their metabolic BW ($BW^{.75}$). All procedures were approved by the University Animal Care Committee.

Statistical Analysis. Data were analyzed using the GLM procedure of SAS (1990). Pig served as the experimental unit. The model included square, period, phytase, diet type, and the interaction of phytase and diet type. Differences between diet type means were tested, using the Duncan's multiple range procedure of SAS.

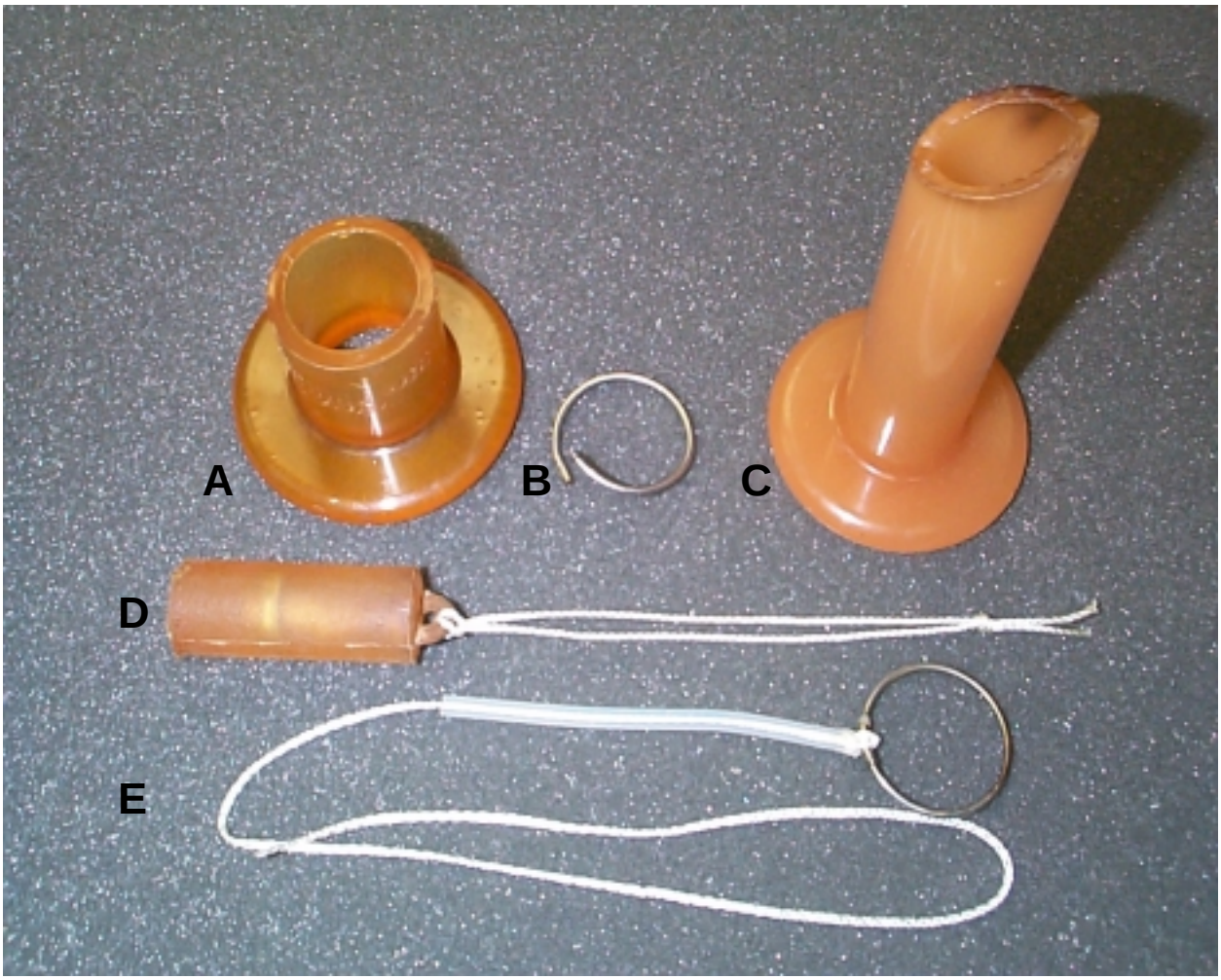

Figure 1. Parts of the steered ileo-cecal valve cannulation system: A) an outer cannula barrel (barrel: 43 mm length, 33 mm i.d., 41 mm o.d.; flange: 80 mm o.d.) B) external ring (2.0 mm thick and 34 mm o.d.) C) inner cannula barrel (barrel: 100 mm length, 26 mm i.d.; flange: 70 mm o.d.) D) cylindrical stopper (26 mm o.d. and 55 mm length) E) internal ring (2.0 mm thick and 35.0 mm inner diameter) attached to a nylon cord.

Results

Dietary Composition. Analyzed dietary composition is shown in Table 4 and was similar to the predicted dietary compositions shown in Table 3, except CP was somewhat lower than calculated. The amount of added phytase was lower than predicted which could be due to a lower activity of the added phytase than predicted for diets 2, 4 and 6 or to degradation of phytase during storage. Diets formulated to contain 500 U of added phytase per kilogram of diet contained an average of only 319 U/kg of diet. Corn-soybean meal based diets and corn-soybean meal-meat meal based diets contained no detectable levels of endogenous phytase activity. In contrast, corn-soybean meal-wheat midds based diets contained approximately 250 U of endogenous phytase activity per kg of diet. This diet contained 20 % wheat middlings which are known to contain high levels of plant phytase activity. Pointilliart (1994) reported that wheat middlings contained an average of 1900 U of phytase activity per kilogram of diet. Based on this value, the CSWM diet should have contained 380 U of phytase activity per kilogram of diet. The assayed value of phytase for this diet was 250 which is 34.2% lower than the calculated value. This reduced value is in agreement with the 36% reduction in assayed versus calculated phytase activity that was observed for diets formulated to contain 500 U of phytase per kilogram of diet. Phytate levels were .73, .86, and .64 % for the corn-soybean, corn-wheat midds, and corn-meat meal based diets, respectively. By multiplying the phytate content of each diet by the percent of P in phytate (28%) the phytate-P content of each diet can be calculated. Based on these calculation the CSWM based diet contained the most phytate P (.24% phytate-P), followed by the CS based diet (.20 % phytate-P), and the CSMB based diet (.18 % phytate-P).

Table 4. Analyzed dietary composition.

	Diet					
Item	1	2	3	4	5	6
CP, %	11.90	11.99	12.35	12.09	12.39	12.42
N, %	1.95	1.93	2.02	2.02	1.98	2.11
DM, %	88.13	88.18	88.20	88.41	88.20	88.12
Ca, %	0.5	0.52	0.46	0.5	0.55	0.57
Cr, %	0.023	0.022	0.026	0.025	0.022	0.023
P, %	0.411	0.415	0.430	0.417	0.475	0.498
Phytase, U/kg	ND	330	251	586	ND	293
Phytate, %	.73	.73	.86	.86	.64	.64
Asp, %	1.10	1.11	1.10	1.06	1.01	1.01
Thr, %	0.46	0.46	0.46	0.44	0.45	0.44
Ser, %	0.57	0.57	0.56	0.53	0.52	0.50
Glu, %	2.15	2.18	2.35	2.31	2.04	2.05
Pro, %	0.83	0.83	0.88	0.88	0.97	0.99
Gly, %	0.51	0.51	0.55	0.54	0.80	0.82
Ala, %	0.69	0.70	0.69	0.68	0.81	0.82
Cys, %	0.25	0.25	0.27	0.28	0.25	0.29
Val, %	0.57	0.58	0.62	0.62	0.60	0.60
Met, %	0.21	0.22	0.22	0.22	0.26	0.26
Ile, %	0.46	0.47	0.50	0.49	0.45	0.45
Leu, %	1.15	1.16	1.13	1.11	1.15	1.16
Tyr, %	0.39	0.39	0.40	0.37	0.36	0.36
Phe, %	0.58	0.59	0.60	0.59	0.55	0.56
His, %	0.34	0.33	0.35	0.34	0.32	0.32
Lys, %	0.61	0.61	0.61	0.59	0.66	0.63
Arg, %	0.75	0.74	0.79	0.77	0.74	0.71

Growth Performance. The addition of microbial phytase to the diet resulted in increased ADG ($P < .05$) across all diet types (Table 5). Pigs that consumed diets containing phytase grew an average of 44 g more per day. The ADG across all diets for the duration of the trial was approximately 500 g/d. Feed efficiency as measured by the gain:feed ratio was also improved ($P = .056$) by phytase supplementation. Gain:feed measured over the length of the trial averaged .293 for diets without phytase and .317 for diets containing phytase. This improvement in feed efficiency with phytase supplementation to the diet was responsible for the improvements seen in growth rate since the pigs were limit fed. Diet type did not affect growth performance or feed efficiency of pigs in this trial.

Apparent Total Tract Digestibilities. Apparent total tract digestibilities (ATTD) of Ca and P were improved by the addition of microbial phytase to the diet (Table 5). The ATTD of Ca and P were improved 6.0 and 9.5 percentage units, respectively, when phytase was added to the diet. By multiplying the percent improvement (9.5%) by the average P content (4.4 g/kg) of the diets, the amount of P released by phytase can be calculated. Based on the results of this study, .42 g of P was released when phytase was added to the diet. Based on a previous report by Kornegay et al. (1998), approximately 76% of the P from inorganic P sources is available to the pig for absorption. Therefore, the P equivalency value of microbial phytase can be calculated by dividing .42 by .76. Based on this calculation, and using the average analyzed added phytase content of the feed, it can be concluded that 319 U of phytase/kg of diet is equivalent to .55 g of P from an inorganic P source.

Table 5. The effects of microbial phytase addition to corn-soybean meal, corn-soybean meal-wheat midds, or corn-soybean meal-meat meal based diets on growth performance and fecal mineral digestibilities[a,b].

Item	Phytase, U/kg	Diet type			**Mean**
		CS[c]	CSWM[d]	CSMB[e]	
ADG, g[f]					
	0	451	493	488	**477**
	500	527	523	514	**521**
	Mean	**489**	**508**	**501**	
Gain:feed[g]					
	0	.271	.307	.302	**.293**
	500	.323	.315	.313	**.317**
	Mean	**.297**	**.311**	**.308**	
Ca digestibility, %[h,i]					
	0	56.27	55.18	65.36	**58.94**
	500	63.75	60.34	70.69	**64.93**
	Mean	**60.01[y]**	**57.76[y]**	**68.03[x]**	
P digestibility, %[h,i]					
	0	46.85	43.93	60.35	**50.38**
	500	58.76	53.20	67.77	**59.91**
	Mean	**52.80[y]**	**48.56[z]**	**64.06[x]**	
DM digestibility, %[i]					
	0	89.38	88.21	89.99	**89.19**
	500	89.77	88.21	89.21	**89.07**
	Mean	**89.58[x]**	**88.21[y]**	**89.60[x]**	

Table 5. (Continued) The effects of microbial phytase addition to corn-soybean meal, corn-soybean meal-wheat midds, or corn-soybean meal-meat meal based diets on growth performance and fecal mineral digestibilities[a,b].

Item	Phytase, U/kg	Diet type			**Mean**
		CS[c]	CSWM[d]	CSMB[e]	
Energy digestibility, %[j]					
	0	88.15	87.45	88.16	**87.97**
	500	87.61	87.12	88.11	**87.61**
	Mean	**87.86[x,y]**	**87.27[y]**	**88.14[x]**	

[a]Each treatment mean represent 12 pigs.
[b]Diet type means within the same row that have different superscripts are different (P < .05).
[c]CS = corn-soybean meal based diet.
[d]CSWM = corn-soybean meal-wheat midds based diet.
[e]CSMB = corn-soybean meal-meat and bone meal based diet.
[f]Phytase effect (P < .05)
[g]Phytase effect (P = .056)
[h]Phytase effect (P < .001)
[i]Diet type effect (P < .001)
[j]Diet type effect (P < .05)

The ATTD of Ca ($P < .001$), P ($P < .001$), DM ($P < .001$), and energy ($P < .01$) were affected by diet type. Pigs fed the CSMB based diet had higher ATTD of Ca, compared to pigs fed either the CS based or the CSWM based diet. Phosphorus ATTD was highest for pigs fed the CSMB based diet and lowest for pigs fed the CSWM based diet. Dry matter ATTD was slightly higher for pigs fed the CSMB and the CS based diets, compared to the CSWM based diet. Finally, the ATTD of energy was higher for pigs fed the CSMB based diet compared to pigs fed the CSWM based diet (Table 5).

Apparent Ileal Digestibilities of Amino Acids and Nitrogen. The addition of microbial phytase to any of the three diet types did not affect the apparent ileal digestibility (AID) of any of the amino acids measured (Table 6). Likewise, apparent ileal N digestibility was not affected by the addition of microbial phytase to the diet.

The AID of all amino acids measured, with the exception of Cys, Val, Ile, Phe, and Arg, were affected by diet type (Table 6). The AID of Asp was highest for pigs fed the CS based diet and lowest for the CSMB based diet. Pigs fed the CS based diet had higher AID of Ser, compared to pigs fed the CSWM or the CSMB based diets. Glutamine and His AID were higher for pigs fed CS and CSWM based diets, compared to pigs fed CSMB based diets. Finally, pigs fed the CS based diet had a higher AID of Leu and Lys compared to pigs fed the CSWM based diet, and a higher AID of Tyr, compared to pigs fed the CSMB based diet. In contrast, pigs fed the CS based diet had a lower AID of Pro compared to pigs fed CSWM and CSMB based diets. Pigs fed the CSMB based diet also had higher AID of Gly, Ala, and Met compared to pigs fed CS or CSWM based diets, and a higher AID of Lys compared to pigs fed the CSWM based diet.

Table 6. The effects of microbial phytase addition to corn-soybean meal, corn-soybean meal-wheat midds, or corn-soybean meal-meat meal based diets on apparent ileal amino acid digestibilities[a,b].

		Diet type			
Amino acid	Phytase, U/kg	CS[a]	CSWM[b]	CSMB[c]	**Mean**
Asp[f]					
	0	81.51	79.71	78.15	**79.79**
	500	82.05	79.50	78.68	**80.08**
	Mean	**81.78[x]**	**79.60[y]**	**78.41[z]**	
Thr[g]					
	0	74.02	71.88	72.92	**72.94**
	500	74.45	71.57	73.17	**73.06**
	Mean	**74.24[x]**	**71.73[y]**	**73.04[x,y]**	
Ser[f]					
	0	83.42	81.27	80.76	**81.82**
	500	83.61	81.04	81.15	**81.94**
	Mean	**83.52[x]**	**81.16[y]**	**80.96[y]**	
Glu[f]					
	0	86.99	87.23	85.27	**86.50**
	500	87.37	87.40	85.40	**86.72**
	Mean	**87.18[x]**	**87.32[x]**	**85.34[y]**	
Pro[h]					
	0	74.48	77.01	77.74	**76.41**
	500	73.28	77.25	76.80	**75.78**
	Mean	**73.88[y]**	**77.13[x]**	**77.27[x]**	
Gly[f]					
	0	71.13	71.36	77.91	**73.46**
	500	70.94	70.69	78.17	**73.27**
	Mean	**71.03[y]**	**71.02[y]**	**78.04[x]**	
Ala[f]					
	0	77.13	75.42	79.40	**77.31**
	500	78.03	75.85	78.99	**77.62**
	Mean	**77.58[y]**	**75.63[z]**	**79.19[x]**	
Cys					
	0	79.98	80.04	80.47	**80.16**
	500	79.97	79.53	80.28	**79.92**
	Mean	**79.98**	**79.78**	**80.37**	

Table 6. (Continued) The effects of microbial phytase addition to corn-soybean meal, corn-soybean meal-wheat midds, or corn-soybean meal-meat meal based diets on apparent ileal amino acid digestibilities[a,b].

		Diet type			
Amino acid	Phytase, U/kg	CS[a]	CSWM[b]	CSMB[c]	**Mean**
Val					
	0	78.75	79.13	79.44	**79.11**
	500	79.16	79.12	79.19	**79.16**
	Mean	**78.96**	**79.12**	**79.31**	
Met[f]					
	0	83.61	82.80	84.56	**83.66**
	500	84.09	83.21	84.95	**84.08**
	Mean	**83.85[y]**	**83.00[y]**	**84.75[x]**	
Ile					
	0	80.54	80.77	80.03	**80.45**
	500	81.03	80.75	79.94	**80.57**
	Mean	**80.79**	**80.76**	**79.99**	
Leu[h]					
	0	84.48	83.72	84.33	**84.18**
	500	84.86	83.73	84.33	**84.31**
	Mean	**84.67[x]**	**83.72[y]**	**84.33[x,y]**	
Tyr[g]					
	0	82.21	81.25	80.45	**81.30**
	500	82.50	81.48	80.36	**81.44**
	Mean	**82.35[x]**	**81.36[x,y]**	**80.40[y]**	
Phe					
	0	84.06	83.78	83.58	**83.81**
	500	84.51	83.87	83.49	**83.96**
	Mean	**84.28**	**83.82**	**83.54**	
His[g]					
	0	86.56	86.63	84.95	**85.84**
	500	86.25	85.67	84.69	**85.54**
	Mean	**86.40[x]**	**85.85[x]**	**84.82[y]**	
Lys[f]					
	0	83.01	81.23	82.76	**82.33**
	500	83.61	81.57	82.54	**82.57**
	Mean	**83.31[x]**	**81.40[y]**	**82.65[x]**	

Table 6. (Continued) The effects of microbial phytase addition to corn-soybean meal, corn-soybean meal-wheat midds, or corn-soybean meal-meat meal based diets on apparent ileal amino acid digestibilities[a,b].

		Diet type			
Amino acid	Phytase, U/kg	CS[a]	CSWM[b]	CSMB[c]	**Mean**
Arg					
	0	89.09	88.60	88.42	**88.70**
	500	88.82	88.50	88.44	**88.58**
	Mean	**88.95**	**88.55**	**88.43**	
Total					
	0	82.09	81.62	81.68	**81.80**
	500	82.33	81.62	81.65	**81.88**
	Mean	**82.21**	**81.62**	**81.68**	
N					
	0	76.20	75.46	76.23	**75.97**
	500	76.53	75.46	76.42	**76.14**
	Mean	**76.37**	**75.46**	**76.33**	

[a]Each treatment mean represent 12 pigs.
[b]Diet type means within the same row that have different superscripts are different (P < .05).
[c]CS = corn-soybean meal based diet.
[d]CSWM = corn-soybean meal-wheat midds based diet.
[e]CSMB = corn-soybean meal-meat and bone meal based diet.
[f]Diet type effect (P < .001)
[g]Diet type effect (P < .005)
[h]Diet type effect (P < .1)

Apparent Ileal Digestibilities of Calcium, Phosphorus, and Dry Matter. There was an overall improvement in the AID of Ca ($P < .001$) when phytase was added to the diet (Table 7). This overall effect was due to a significant improvement in the AID of Ca when phytase was added to all three diets. The AID of P was also improved when phytase was added to the diet ($P < .001$). The effect on the AID of P, like that on the AID of Ca, was seen within each diet type. There were no effects of supplementary microbial phytase on the AID of DM.

Calcium ($P < .001$), P ($P < .001$), and DM ($P < .001$) AID were also affected by diet type (Table 7). Calcium AID was highest for pigs fed the CSMB based diet, and was similar for pigs fed either the CS based diet or the CSWM based diet. Phosphorus AID was highest for pigs fed the CSMB based diet and lowest for pigs fed the CSWM based diet. Finally, DM AID was lowest for pigs fed the CSWM based diet and was similar for pigs fed the CS or the CSMB based diet.

Discussion

The addition of microbial phytase to pig diets has been shown to increase the ATTD of P (Jongbloed et al., 1992; Cromwell et al., 1993; Lei et al., 1993b, Kornegay et al., 1995, 1996; Jongbloed, 1996; Radcliffe and Kornegay, 1998; Skaggs, 1999; Rice et al., 1999; Zhang, 1999; Rice et al., 2000; Robbins et al., 2000). The efficacy of microbial phytase has been shown to differ between diet types. Factors in the diet which affect the efficacy of microbial phytase include the amount of phytate P, the level of endogenous phytase activity, the amount of

Table 7. The effects of microbial phytase addition to corn-soybean meal, corn-soybean meal-wheat midds, or corn-soybean meal-meat meal based diets on apparent ileal Ca, P and DM digestibility[a,b].

Item	Phytase, U/kg	Diet type			**Mean**
		CS[c]	CSWM[d]	CSMB[e]	
Ca[f,g]					
	0	63.92	62.39	69.01	**65.11**
	500	68.78	68.31	74.96	**70.68**
	Mean	**66.35[y]**	**65.35[y]**	**71.98[x]**	
P[f,g]					
	0	48.07	44.01	60.51	**50.86**
	500	60.48	58.40	70.38	**63.09**
	Mean	**54.27[y]**	**51.20[z]**	**65.44[x]**	
DM[g]					
	0	77.51	74.16	76.67	**76.11**
	500	77.28	74.13	77.99	**76.47**
	Mean	**77.39[x]**	**74.15[y]**	**77.33[x]**	

[a]Each treatment mean represent 12 pigs.
[b]Diet type means within the same row that have different superscripts are different (P < .05).
[c]CS = corn-soybean meal based diet.
[d]CSWM = corn-soybean meal-wheat midds based diet.
[e]CSMB = corn-soybean meal-meat and bone meal based diet.
[f]Phytase effect (P < .001)
[g]Diet type effect (P < .001)

inorganic P, and the amount of Ca (Jongbloed et al., 1996; Kornegay et al., 1996; Veum, 1996; Qian et al., 1996a,b).

This study investigated three diet types: CS, CSWM, and CSMB based diets. Since the the majority of published reports investigating the addition of microbial phytase to swine diets has been in pigs fed CS based diets, it seems logical to compare the results with the CS diet in this trial against previous published results, and then to compare to CSWM and the CSMB based diets with the CS based diet used in this study. An increase in ADG was observed across all three diet types when phytase was added to the diet, as has been observed in previous findings (Simons et al., 1990; Beers and Jongbloed, 1992; Jongbloed et al., 1992; Kornegay and Qian, 1996; Yi et al., 1996c). Since pigs in this study were limit fed at a level of 9% of metabolic BW ($BW^{.75}$), this increase in ADG was due to an increased feed efficiency as has been reported in previous studies (Simons et al., 1990; Beers and Jongbloed, 1992; Jongbloed et al., 1992; Kornegay and Qian, 1996; Yi et al., 1996c). The ATTD of P was increased 11.91 percentage units when 319 U (analyzed) of phytase were added per kilogram of diet. By multiplying this increase by the amount of P in the diet (.44%) it can be concluded that 319 U of phytase added per kilogram of diet releases .052% or .52 g of P per kilogram of diet. If the amount of released P is divided by the bioavailability of inorganic P sources (76.7%) as estimated by Kornegay et al. (1998), then it can be concluded that 319 U of phytase added per kilogram of diet is equivalent to .68 g of P from inorganic P per kilogram of diet. This value is in agreement with those reported in the literature where it was found that 500 U of phytase per kilogram of diet was equivalent to approximately 1 g of P from inorganic P per kilogram of diet. The relatively high basal level of P included in this diet (.44%) compared with much of the data reported in the literature where no

inorganic P was added may have affected the efficacy of phytase. A typical CS based diet with no added inorganic P would contain approximately .35% P. However, the value of .68 g of P is higher than that reported in Chapter IV where 500 U of phytase per kilogram of a CS based diet was calculated to replace .59 g of P from inorganic phosphate.

The ATTD of Ca was improved 7.48 percentage units when 319 U (analyzed) of phytase were added per kilogram of diet to the CS based diet. If this increase in Ca ATTD is multiplied times the amount of Ca in the diet (.50%) then it can be concluded that 319 U of phytase added per kilogram of diet can release .037% or .37 g of Ca per kilogram of diet. Radcliffe (1997) estimated that Ca from $CaCO_3$ was 82% available. Based on this estimate, .45 g of Ca can be released per kilogram of diet when 319 U of phytase are added per kilogram of diet. The dietary level of Ca (.50%) in this study was at or above the requirement of pigs and therefore may have decreased the impact of microbial phytase on Ca digestibility.

In contrast to the results reported in Chapter IV, no improvements in the AID of amino acids were observed when phytase was added to the diet. This was true for all diet types. Pigs fed the CS based diet had a higher AID of all amino acids, except proline, compared with pigs fed the 12% CP CS based diet in chapter IV (Table 8). This higher AID has two possible explanantions. First, the pigs in this study were able to utilize a larger percent of the plant derived amino acids than those in Chapter IV, or second, the higher CP content of the CS diet in this study compared to Chapter IV (13% vs. 12%) resulted in a smaller proportion of endogenous N loss in the collected ileal digesta and therefore a higher AID of amino acids. Based on the results of chapter IV, it does appear that endogenous N loss as a percent of ileal N does affect the

Table 8. Comparison of amino acid digestibility values calculated from Chapter IV and from the present study.

	Apparent ileal digestibilities, %	
Amino acid	Chapter IV[a]	Present study[b]
Glycine	69.7	71.0
Alanine	76.1	77.6
Valine	77.4	79.0
Leucine	82.8	84.7
Isoleucine	79.5	80.8
Proline	79.0	73.9
Serine	81.0	83.5
Threonine	72.3	74.2
Cystine	79.0	80.0
Methionine	79.8	83.9
Aspartic acid	79.6	81.8
Glutamic acid	85.2	87.2
Tyrosine	78.9	82.4
Phenylalanine	82.4	84.3
Histidine	83.9	86.4
Lysine	79.4	83.3
Arginine	87.1	89.0

[a]Apparent ileal digestibilities from pigs fed the high CP diet (12% CP).

[b]Apparent ileal digestibilities of pigs fed the CS based diet. Values represent an average of pigs fed CS based diets with and without added phytase.

AID of amino acids. However, without a method to quantify endogenous N losses, it remains unclear to what extent this affects the AID of amino acids. It is also possible that the amino acids in SBM are more bioavailable than those in corn. As a result, as the CP content of the diet is increased, so is the AID of N and amino acids.

Previous studies have reported an increase in ileal N or amino acid digestibility when phytase was added to the diet (Mroz et al., 1991; Officer and Batterhan, 1992; Khan and Cole, 1993; Kemme et al., 1995; Jongbloed et al., 1995; Zhang and Kornegay, 1999). However, a number of other studies have found no improvement (Nasi, 1990; Kemme and Jongbloed, 1993a,b,c; Lantzsch and Drochner, 1995). The ability of phytase to increase amino acid AID is affected by the CP level fed, the amount of phytic acid in the diet, the amount of phytase added to the diet, the level of Ca and P in the diet, and many other dietary factors. In this study, the CP level fed was higher than in the experiment reported in Chapter IV, and the assayed level of phytase was much lower than the calculated values. These factors may have contributed to the lack of an affect of phytase on amino acids observed in this study. However, if phytase has the ability to increase amino acid AID, but the effects can only be observed under certain experimental conditions, then it remains uncertain if the effect of phytase on amino acid AID has any practical applications.

Pigs fed the CSMB based diet had higher AID and ATTD of Ca and P compared to pigs fed the CS or CSWM based diets. This is due to the high bioavailability of Ca and P from meat and bone meal which was estimated to be 90% by the swine NRC (1998). Diet type also affected amino acid digestibility, but no diet was consistently better than any other diet. These differences in amino acid AID between diets were undoubtedly due to differences in the amino acid

composition between diet types, and to differences in the phytic acid and endogenous phytase content of the different diet types.

Implications

The results of this study confirm that the addition of microbial phytase to swine diets improves Ca and P AID and ATTD. However, the effects of phytase on the AID of amino acids were not observed in this study. Therefore, further research is needed to determine under what dietary conditions and in what diet types this effect of phytase on amino acid digestibility can be observed.

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Chapter VI

The effects of microbial phytase on mineral, amino acid, and energy digestibilities in grow-finish pigs fitted with steered ileo-cecal valve cannulas and fed corn-wheat-soybean meal, corn-wheat-canola, or corn-sorghum-soybean meal based diets

ABSTRACT. Twelve crossbred barrows fitted with steered ileo-cecal valve cannulas, used in a paired 6 x 6 Latin square design, were fed corn-wheat-soybean meal (CWS), corn-wheat-canola (CWC), or sorghum-corn-soybean meal (SCS) based diets with or without 500 U of added phytase/kg of diet. Each 14-d period consisted of a 7-d adjustment followed by a 3-d total collection, a 12-h ileal digesta collection, a 3-d readjustment, and a second 12-h ileal digesta collection. Pigs were individually housed in metabolism pens (1.2m x 1.2m). Pigs had *ad libitum* access to water, and feed was supplied at a level of 9% of metabolic BW ($BW^{.75}$). The addition of microbial phytase to all diet types improved the apparent total tract digestibility (ATTD) of P (P<.001) and the apparent ileal digestibility (AID) of P (P<.001), Asp, Thr, Ser, Ala, Tyr, Phe, Lys, and Arg (P<.001-.05), and tended to improve Gly AID (P<.1). Amino acid AID was improved an average of 1.47 percentage units. Pigs fed CWC based diets had lower (P<.001) ATTD of P, DM, and energy, compared to pigs fed CWS or SCS based diets. Phosphorus ATTD was higher for pigs fed CWS based diets, compared to pigs fed SCS based diets. Pigs fed the CWS based diets had higher (P<.001) AID of all amino acids measured, compared to pigs fed CWC or SCS based diets. The AID of Glu, Pro, Gly, Cys, Met, and His were lower (P<.001) for pigs fed the SCS based diets compared to the CWC based diets. An interaction of phytase and diet type was observed for the ATTD of Ca (P<.02), and the AID of Ca (P<.1), Met (P<.05), and Val (P<.01). The AID and ATTD of Ca were improved in all diet types by the addition of phytase, but the magnitude of the response was greater in the SCS based

diets. The AID of Met and Val was improved when phytase was added to the CWS and CWC based diets, but decreased when phytase was added to the SCS based diet.

Key Words: Pigs, Phytase, Amino acids, Canola, Minerals, Energy

Introduction

Environmental concern over the P content of swine waste has caused nutritionists to focus on methods of lowering fecal P excretion. One of the best nutritional tools for lowering fecal P is microbial phytase. Phytase releases phytate P, that is normally unavailable to the pig, from the phytate molecule, thereby increasing the digestibility of plant P. Several studies designed to investigate the effects of microbial phytase on P digestibility in pigs have reported an increased N digestibility. Each phosphate group of phytate can carry one to two negative charges at a neutral pH. As a result, the phytate molecule has the potential to form complexes with positively charged minerals, amino acids and/or peptides, and sugars (Figure 1). Nitrogen in swine waste also poses environmental concerns and it is a costly dietary ingredient, as high protein feed ingredients are generally more expensive. As a result, any improvements in amino acid digestibility when phytase is added to the diet could improve the economic efficiency of adding phytase to the ration. In several studies the effects of phytase on amino acid digestibilities have been investigated. The majority of these have added phytase to corn-soybean meal based diets. The effectiveness of phytase for improving the digestibility of P in the diet has been shown to be affected by the amount of phytate P in the diet, the level of total Ca and P in the diet, the amount of endogenous phytase present in the diet, and the Ca to P ratio in the diet (Jongbloed et al., 1996; Kornegay et al., 1996; Veum, 1996; Qian et al., 1996a, b). It seems reasonable that these factors, in addition to the total CP level of the diet, the amino acid composition of the diet, and the amino acid ratios in the diet would affect the efficiency of phytase for improving the digestibility of dietary amino acids. Hence, phytase efficacy for improving amino acid

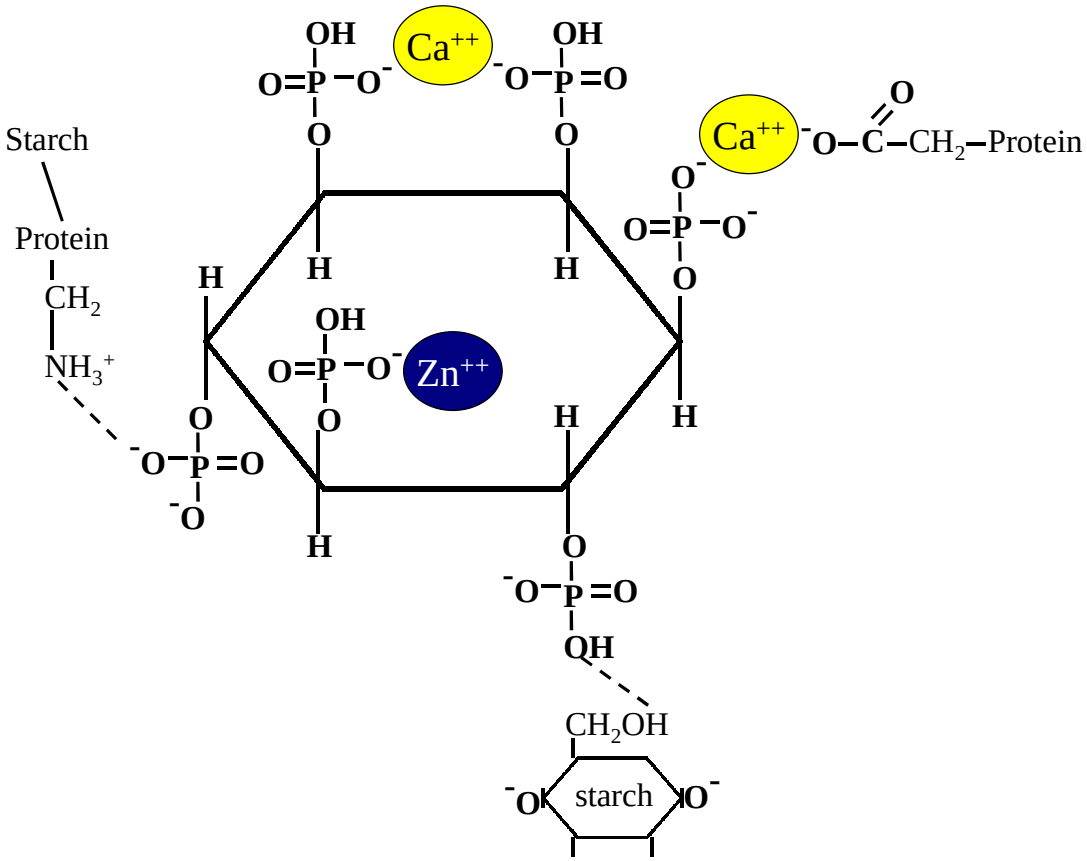

Figure 1. Possible interactions with of minerals, starch and amino acids with the phytate molecule.

digestibilities would differ for different diet types. Therefore, this study was designed primarily to investigate the effects of phytase on the apparent ileal digestibility of amino acids from corn-wheat-soybean meal, corn-wheat-canola, and corn-sorghum-soybean meal based diets. Effects on mineral and energy digestibility were studied also.

Materials and Methods

Animals, Housing, Experimental Design, and Analysis. Twelve crossbred barrows fitted with steered ileo-cecal valve (SICV) cannulas (described in Chapter III) were used in a Latin square design (Table 1) to test the effects of added phytase on mineral, amino acid, and energy digestibilities. Dietary treatments are shown in Table 2 and the composition of the basal diets are shown in Table 3. Diets 1 and 2 were corn-wheat-soybean meal based (CWS), diets 3 and 4 were corn-wheat-canola based (CWC), and diets 5 and 6 were sorghum-corn-soybean meal (SCS) based. Microbial phytase (Natuphos 600) was added at a level of 500 U/kg to diets 2, 4 and 6. Pigs were individually housed in metabolism pens (1.2 m x 1.2 m). Water was supplied ad libitum and feed was supplied at a level of 9% of metabolic BW ($BW^{.75}$) per day in two daily feedings (0800 h and 1800 h). All diets contained .05% chromic oxide as an indigestible marker to allow for calculation of digestion coefficients.

Procedures for insertion of the SICV cannulas were as described in Chapter 3. Breifly, the SICV cannula consists of 5 parts (Figure 2): an inner cannula barrel (barrel, 100 mm length, 26 mm i.d.; flange, 70 mm o.d.), an outer cannula barrel (barrel, 43 mm length, 33 mm i.d., 41

Table 1. Experimental design.

	Pen no., block 1					
Period	**1**	**2**	**3**	**4**	**5**	**6**
1	1[a]	2	3	4	5	6
2	6	1	2	3	4	5
3	2	3	4	5	6	1
4	5	6	1	2	3	4
5	3	4	5	6	1	2
6	4	5	6	1	2	3

	Pen no., block 2					
Period	**7**	**8**	**9**	**10**	**11**	**12**
7	1	2	3	4	5	6
8	6	1	2	3	4	5
9	2	3	4	5	6	1
10	5	6	1	2	3	4
11	3	4	5	6	1	2
12	4	5	6	1	2	3

[a]Diets

Table 2. Dietary treatments.

	Diet					
Item	1	2	3	4	5	6
Basal	CWS[a]	CWS[a]	CWC[b]	CWC[b]	SCS[c]	SCS[c]
Corn starch (%)	.083	---	.083	---	.083	---
Phytase (U/kg)	---	500	---	500	---	500

[a]CWS = corn-wheat-soybean meal based diet.
[b]CWC= corn-wheat-canola meal based diet.
[c]SCS = sorghum-corn-soybean meal based diet.

Table 3. Composition of basal diets.

	Diet			NRC Requirement, %	
Item	1 & 2 CWS[a]	3 & 4 CWC[b]	5 & 6 SCS[c]	50-80 kg	80-120 kg
Ingredient composition, %					
Corn	47.45	43.16	15.73		
Wheat	40.00	40.00	0.00		
Sorghum	0.00	0.00	69.90		
Canola meal	0.00	14.94	0.00		
Limestone[d]	1.01	0.85	0.84		
Lysine HCl	0.08	0.13	0.08		
SBM	10.51	0.00	12.21		
Salt	0.34	0.32	0.35		
DiCal[e]	0.03	0.01	0.31		
Vit. Premix[f]	0.20	0.20	0.20		
TM premix[g]	0.05	0.05	0.05		
Se premix[h]	0.05	0.05	0.05		
Cr-premix[i]	0.20	0.20	0.20		
Empty space[j]	0.08	0.08	0.08		
Calculated composition					
CP, %	13.50	13.50	13.50	15.5	13.2
Lys, %	0.64	0.66	0.63	.75	.60
Ileal digestible Lys, %	0.51	0.51	0.50	.61	.47
Met, %	0.23	0.26	0.23	.20	.16
Ileal digestible Met, %	0.20	0.22	0.19	.17	.13
Trp, %	0.16	0.15	0.16	.14	.11
Ileal digestible Trp, %	0.12	0.11	0.12	.10	.08
Thr, %	0.48	0.51	0.49	.51	.41
Ileal digestible Thr, %	0.35	0.36	0.36	.37	.30
ME (kcal/kg)	3303.19	3250.00	3288.39	3265	3265
Ca, %	0.45	0.45	0.45	.50	.45
P, %	0.36	0.43	0.38	.45	.40
Available P, %	0.12	0.13	0.12	.19	.15
Na, %	0.15	0.15	0.15	.10	.10
Crude fat, %	2.54	3.80	3.01	---	---
NDF, %	10.89	12.63	15.18	---	---
ADF, %	3.50	5.40	6.90	---	---

[a]CWS = corn-wheat-soybean meal based diet.
[b]CWC= corn-wheat-canola meal based diet.
[c]SCS = sorghum-corn-soybean meal based diet.
[d]Ground limestone had a guaranteed minimum analysis of 96% $CaCO_3$ (Roanoke City Mills, Roanoke, VA).

[e]Dicalcium phosphate (PCS Phosphate Company, Inc., Raleigh, NC; guaranteed analysis: 18.5% P min., 20.0% Ca min., 24.0% P max.
[f]Supplied per kilogram of diet: retinyl acetate, 1211 μg; cholecalciferol, 88 μg; dl-α-tocopherol acetate, 18 mg, riboflavin, 3.5 mg; niacin, 18 mg; choline chloride, 352 mg; d-pantothenic acid, 18 mg; d-biotin, 0.35 mg; cyancobalamin, 18 μg; menadione dimethylprimidinol bisulfate, 1.8 mg.
[g]Supplied per kilogram of diet: Zn, 75 mg; Fe, 88 mg; Mn, 30 mg; Cu 8 mg; I, 2 mg.
[h]Supplied 0.3 mg Se per kilogram of diet
[i]Chromic oxide premix = a mixture of Cr_2O_3 and corn starch in a ration of 1 part Cr_2O_3 to 3 parts corn starch on a wt:wt basis. Added to supply .05% Cr_2O_3 to the diet.
[j]Empty space was filled with corn starch or phytase in individual diets.

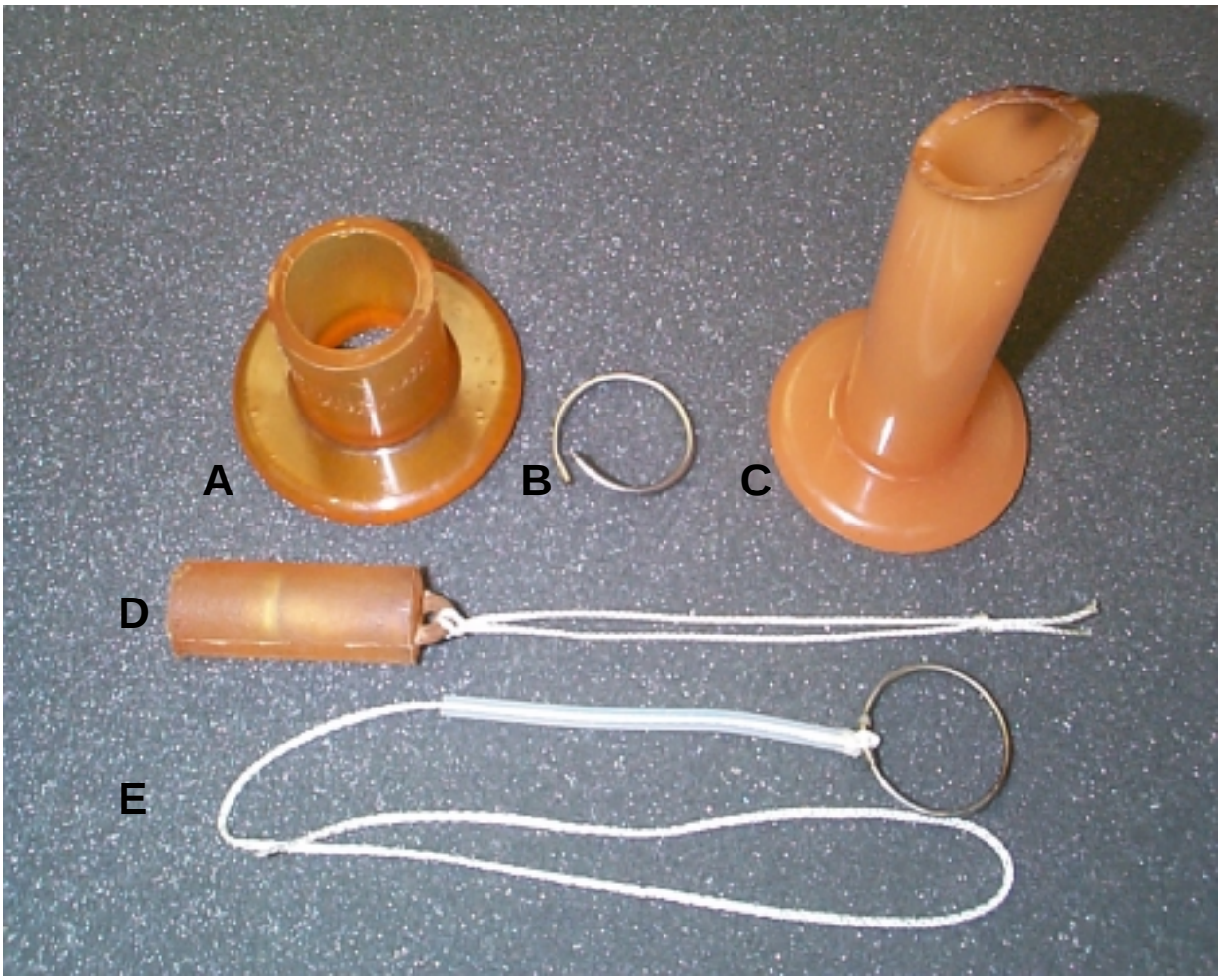

Figure 2. Parts of the steered ileo-cecal valve cannulation system: A) an outer cannula barrel (barrel: 43 mm length, 33 mm i.d., 41 mm o.d.; flange: 80 mm o.d.) B) external ring (2.0 mm thick and 34 mm o.d.) C) inner cannula barrel (barrel: 100 mm length, 26 mm i.d.; flange: 70 mm o.d.) D) cylindrical stopper (26 mm o.d. and 55 mm length) E) internal ring (2.0 mm thick and 35.0 mm inner diameter) attached to a nylon cord.

mm o.d.; flange, 80 mm o.d.), an internal ring (2.0 mm thick and 35.0 mm inner diameter) attached to a nylon cord, an external ring (2.0 mm thick and 34 mm o.d.), and a cylindrical stopper (26 mm o.d. and 55 mm length).

Feed was withheld from pigs for 36 h prior to surgery and water was removed 12 h prior to surgery. Anesthesia was induced with an i.m. injection of medetomadine (80 μg/kg BW), ketamine (10 mg/kg BW), and butorphanol (10 mg/kg BW), and pigs were maintained on halothane in oxygen via an endotracheal tube for the duration of the surgical procedure. Prior to surgery pigs were given i.v. injections of Naxcel® (1 mg/lb BW) and banamine (.5 mg/lb BW). Surgical procedures for the insertion of the SICV cannula were as described in Chapter III. On the day following surgery pigs were fed 50 g of feed which was increased 100 g/d up to a level of 9% of their metabolic BW ($BW^{.75}$).

Each 2-wk period consisted of a 7-d adjustment period followed by a 7-d collection period that consisted of a 3-d total collection, a 12-h ileal digesta collection, a 3-d readjustment period and a second 12-h ileal digesta collection. During the 12-h ileal collection, digesta was emptied out of the collection bags and placed on dry ice as soon as it appeared, and was weighed and placed in an ultra low temperature freezer (-80°C) every hour. Total excretions of feces and urine were collected separately during each 3-d total collection. Feces were collected by placing a plastic bag over the anus of each pig following the procedures of Van Kleef et al. (1994). Urine was collected in buckets from the drop pans under each pen. A 25% HCl solution was added to each bucket to maintain a urine pH of less than 5. Total urine and feces were collected twice per day during the collection periods and frozen at -20°C for subsequent analysis. Pigs

were weighed every 2 wk prior to the start of the next period at which time feeding levels were adjusted.

Fecal samples were dried in a forced air oven at 60°C and ileal samples were freeze dried. Diet, fecal, and ileal digesta samples were ground to pass through a 1 mm screen and analyzed for Ca, P, and Cr following nitric-perchloric acid (5:3, vol:vol) wet digestion. Total P concentrations were assayed photometrically using the vanadomolybdate procedure (AOAC, 1990) and Ca and Cr were determined using an atomic absorption spectrophotometer (model 5100 PC, Perkin-Elmer, Norwalk, CT) following the manufacturer's recommended procedures. Samples were analyzed for N content using AOAC (1990) methods. Diets and ileal digesta were hydrolyzed in 6 N HCl for 24 h and analyzed for amino acid content using HPLC by the Experiment Station Chemical Laboratories at the University of Missouri-Columbia (Columbia, MO 65211). A bomb calorimeter (Parr Model 1271 with automatic bomb handling and module with built-in bomb, Parr Instrument Company, Moline, IL) was used to determine gross energy of feed and fecal samples.

Statistical Analysis. Data were analyzed using the GLM procedure of SAS (1990) using pig as the experimental unit. The model included square, period, phytase, diet type, and the interaction of phytase and diet type. Differences between diet type means were tested using the Duncan's multiple range procedure of SAS.

Results

Dietary Composition. Analyzed dietary composition (Table 4) was similar to the calculated composition (Table 3). However, analyzed CP content was generally lower than calculated values except for diets 5 and 6. Diets containing wheat had a slightly lower CP content than predicted which was most likely due to an overestimation of the protein content of wheat. Assayed phytate content of the diets was highest for diets containing canola meal. This was expected since canola has a higher phytate content then any of the other plant feed ingredients used in this study. Since phytate contains approximately 28% P, the phytate P content can be calculated by multiplying the assayed phytate content of the feed by .28. This yields a phytate P content of .24, .32, and .23%, respectively for CWS, CWC, and SCS based diets. The wheat provided approximately 420 U of phytase activity/kg of diet. Therefore, the addition of phytase to the wheat based diets increased the assayed level of phytase to over 920 U/kg of diet. In contrast, the SCS based diet provided no detectable endogenous phytase activity. Therefore, the phytase supplemented SCS based diet contained only 410 U/kg of phytase.

Growth Performance. Average daily gain was not affected by the addition of phytase or by diet type (Table 5). Pigs gained an average of 503 g/d during the 12-wk trial. Feed efficiency, as measured by the gain:feed ratio was also not affected by phytase addition or diet type. The average gain:feed ratio for the 12-wk trial was .306.

Apparent Total Tract Digestibilities. An interactive effect ($P < .02$) of phytase and diet type was observed (Table 5) for Ca apparent total tract digestibility (ATTD). Only slight

Table 4. Analyzed dietary composition.

	Diet					
Item	1	2	3	4	5	6
CP, %	12.31	12.72	12.37	12.52	13.97	13.58
N, %	2.17	2.1	2.08	2.12	2.29	2.44
DM, %	88.83	89.24	88.6	88.2	88.83	88.39
Energy, kcal/kg	3957	3989	3958	4036	3949	4011
Ca %	0.481	0.496	0.578	0.573	0.405	0.428
Cr %	0.024	0.024	0.029	0.028	0.027	0.025
P %	0.362	0.367	0.445	0.432	0.4	0.377
Phytase, U/kg	413	944	427	921	ND	410
Phytate, %	.86	.86	1.14	1.14	.81	.81
Tau, %	0.03	0.07	0.06	0.06	0.02	0.17
Asp, %	1.02	1.07	0.84	0.86	1.24	1.2
Thr, %	0.45	0.47	0.48	0.48	0.5	0.5
Ser, %	0.56	0.59	0.54	0.54	0.61	0.63
Glu, %	2.81	2.87	2.84	2.88	2.81	2.69
Pro, %	0.95	0.97	1.01	1.03	0.92	0.95
Gly, %	0.52	0.54	0.57	0.58	0.53	0.5
Ala, %	0.62	0.64	0.63	0.63	1	0.98
Cys, %	0.31	0.31	0.35	0.37	0.28	0.25
Val, %	0.59	0.59	0.61	0.61	0.7	0.61
Met, %	0.25	0.26	0.29	0.29	0.27	0.24
Ile, %	0.46	0.46	0.44	0.44	0.57	0.5
Leu, %	1.06	1.08	1.03	1.03	1.53	1.47
Tyr, %	0.39	0.41	0.37	0.38	0.5	0.5
Phe, %	0.6	0.61	0.56	0.59	0.72	0.7
His, %	0.33	0.34	0.34	0.34	0.35	0.34
Lys, %	0.61	0.64	0.64	0.64	0.64	0.59
Arg, %	0.74	0.77	0.7	0.71	0.77	0.73

Table 5. The effects of microbial phytase addition to wheat-soybean meal, wheat-canola, or sorghum-soybean meal based diets on growth performance and fecal mineral digestibilities[a,b].

Item	Phytase, U/kg	Diet type			Mean
		CWS[c]	CWC[d]	SCS[e]	
ADG, g					
	0	497	502	508	**503**
	500	533	448	530	**504**
	Mean	**515**	**475**	**519**	
Gain:Feed					
	0	.301	.309	.306	**.305**
	500	.328	.268	.321	**.306**
	Mean	**.314**	**.289**	**.313**	
Ca Digestibility, %[f,g,h]					
	0	69.61	57.98	57.19	**61.70**
	500	70.86	59.69	70.74	**67.10**
	Mean	**70.24[x]**	**58.87[z]**	**63.97[y]**	
P Digestibility, %[g,I]					
	0	61.22	46.27	51.55	**53.20**
	500	70.22	52.41	65.96	**62.65**
	Mean	**65.52[x]**	**49.47[z]**	**58.76[y]**	
DM digestibility, %[g]					
	0	93.39	88.30	91.84	**91.26**
	500	93.10	88.15	93.25	**91.50**
	Mean	**93.25[x]**	**88.22[y]**	**92.54[x]**	
Energy digestibility, %[g]					
	0	91.90	86.05	89.37	**89.21**
	500	91.27	85.96	91.24	**89.49**
	Mean	**91.58[x]**	**86.00[y]**	**90.30[x]**	

[a]Each treatment mean represent 12 pigs.
[b]Diet type means within the same row that have different superscripts are different (P < .05).
[c]CWS = corn-wheat-soybean meal based diet.
[d]CWC= corn-wheat-canola meal based diet.
[e]SCS = sorghum-corn-soybean meal based diet.
[f]Phytase effect (P < .002)
[g]Diet type effect (P < .001)
[h]Phytase*Diet type effect (P < .02)
[i]Phytase effects (P < .001)

improvements were observed for Ca ATTD when phytase was added to diets containing wheat, but when phytase was added to the SCS based diet a 13.5 percentage unit increase in Ca ATTD was observed.

The addition of phytase to all diet types resulted in an increased ATTD of P ($P < .001$). On average, P ATTD was increased by 9.4 percentage units when 500 U of phytase per kilogram of diet were added. By multiplying 9.4% by the average P concentration of all diets (3.97 g/kg) the amount of P released by phytase can be calculated (.373 g). If it is assumed that the average availability of P from an inorganic P source is 76%, as reported by Kornegay et al. (1998), then the equivalency value of phytase for P can be calculated by dividing .373 g by 76%. Since all of the diets containing phytase were formulated to contain 500 U of phytase/kg, based on P ATTD alone, 500 U of phytase is equivalent to .49 g of P from an inorganic P source. Dry matter and energy ATTD were unaffected by the addition of phytase.

The ATTD of P, DM, and energy were affected ($P < .001$) by the diet type. Phosphorus ATTD was highest for pigs fed the CWS based diet and lowest for pigs fed the CWC based diet. These differences in P ATTD are logical. The endogenous phytase in the wheat should release some of the phytate P, and thus pigs fed the wheat-soybean meal based diet had the highest P ATTD. This beneficial action of endogenous wheat phytase was overcome in the wheat-canola based diet by the detrimental actions of excess phytate-P as a result of the high phytate content of canola. Dry matter and energy ATTD were also lowest for pigs fed the wheat-canola based diet and were similar for pigs fed the wheat-soybean meal and the sorghum-soybean meal based diets.

Apparent Ileal Digestibility of Amino Acids and Nitrogen. An interaction between phytase addition and diet type was observed for Val ($P < .01$) and Met ($P < .05$) AID. In both

cases, the addition of phytase to the CWS and the CWC led to small increases in the AID of Val and Met, but the addition of phytase to the SCS meal based diet led to a small decrease for Val and Met digestibility (Table 6).

The addition of microbial phytase to all diet types led to increased AID of all amino acids measured ($P < .01$: Asp, Thr, Ser; $P < .05$: Ala, Tyr, Phe, Lys, Arg; $P < .1$: Gly). However, this effect was not significant for Glu, Pro, Cys, Ile, Leu, and His. On the average, amino acid digestibilities were improved by 1.47 percentage units (range: .88 to 1.96 percentage units). The total amino acid AID was also improved ($P < .05$) by 1.13 percentage units. An improvement of 1.72 percentage units was also observed for N AID ($P < .01$).

The type of diet fed affected the AID of all amino acids measured ($P < .001$). The AID of all amino acids, except Ala, was higher for pigs fed CWS based diets compared to pigs fed CWC or SCS based diets. The AID of Ala for pigs fed CWS diets was higher than for pigs fed CWC based diets, but similar to pigs fed SCS based diets. Pigs fed the SCS based diet had the lowest Glu, Pro, Gly, Cys, Met, and His AID. Pigs fed the CWC based diet had the lowest AID of Asp, Thr, Ser, Ile, Tyr, and Lys. Finally, the AID of Val, Leu, Phe, and Arg were similar for pigs fed the CWC based diet and the SCS based diet, but both were significantly lower than those of pigs fed the CWS based diet. Total amino acid AID was also influenced by diet type. Pigs fed the CWC based or the SCS based diets had similar total amino acid AID which were lower ($P < .05$) than those of pigs fed CWS based diets. The AID of N was highest for pigs fed the CWS based diets followed by pigs fed the SCS based diets, and then by pigs fed the CWC based diets ($P <$

Table 6. The effects of microbial phytase addition to wheat-soybean meal, wheat-canola, or sorghum-soybean meal based diets on apparent ileal amino acid digestibilities(%)[a,b].

		Diet type			
Amino acid	Phytase, U/kg	CWS[c]	CWC[d]	SCS[e]	Mean
Asp[f,g]					
	0	82.96	74.22	80.84	**79.34**
	500	85.17	76.52	82.09	**81.26**
	Mean	**84.07[x]**	**75.37[z]**	**81.47[y]**	
Thr[f,g]					
	0	78.44	71.96	73.87	**74.76**
	500	80.78	73.40	75.99	**76.72**
	Mean	**79.61[x]**	**72.68[z]**	**74.93[y]**	
Ser[f,g]					
	0	85.65	79.65	80.54	**81.92**
	500	87.29	80.29	82.74	**83.44**
	Mean	**86.47[x]**	**79.92[z]**	**81.64[y]**	
Glu[g]					
	0	91.66	89.32	85.47	**88.82**
	500	92.48	89.97	86.05	**89.50**
	Mean	**92.07[x]**	**89.64[y]**	**85.76[z]**	
Pro[g]					
	0	88.21	82.74	74.97	**81.98**
	500	89.21	83.13	77.64	**83.32**
	Mean	**88.71[x]**	**82.94[y]**	**76.31[z]**	
Gly[g,h]					
	0	78.79	73.43	70.96	**74.39**
	500	80.66	75.63	72.20	**76.16**
	Mean	**79.72[x]**	**74.53[y]**	**71.58[z]**	
Ala[g,i]					
	0	80.64	76.56	80.27	**79.15**
	500	82.65	77.77	80.93	**80.45**
	Mean	**81.65[x]**	**77.16[y]**	**80.60[x]**	

Table 6. *(Continued) The effects of microbial phytase addition to wheat-soybean meal,wheat-canola, or sorghum-soybean meal based diets on apparent ileal amino acid digestibilities (%)*[a,b].

Amino acid	Phytase, U/kg	Diet type			Mean
		CWS[c]	CWC[d]	SCS[e]	
Cys[g]					
	0	87.06	83.10	78.25	**82.80**
	500	87.83	84.80	77.44	**83.36**
	Mean	**87.45[x]**	**83.95[y]**	**77.84[z]**	
Val[g,j]					
	0	83.84	78.26	80.53	**80.88**
	500	84.60	79.56	78.85	**81.00**
	Mean	**84.22[x]**	**78.91[y]**	**79.69[y]**	
Met[g,k]					
	0	88.17	86.55	84.05	**86.26**
	500	90.05	87.26	83.17	**86.83**
	Mean	**89.11[x]**	**86.90[y]**	**83.61[z]**	
Ile[g]					
	0	84.38	77.96	81.19	**81.18**
	500	85.24	79.07	80.22	**81.51**
	Mean	**84.81[x]**	**78.52[z]**	**80.70[y]**	
Leu[g]					
	0	86.99	82.94	83.81	**84.58**
	500	88.13	83.56	84.07	**85.25**
	Mean	**87.56[x]**	**83.25[y]**	**83.94[y]**	
Tyr[g,i]					
	0	83.94	77.60	80.21	**80.58**
	500	85.67	78.63	81.80	**82.03**
	Mean	**84.81[x]**	**78.11[z]**	**81.00[y]**	
Phe[g,i]					
	0	86.92	82.53	83.37	**84.27**
	500	88.16	84.11	83.84	**85.37**
	Mean	**87.54[x]**	**83.32[y]**	**83.61[y]**	

Table 6. *(Continued) The effects of microbial phytase addition to wheat-soybean meal, wheat-canola, or sorghum-soybean meal based diets on apparent ileal amino acid digestibilities*[a,b].

		Diet type			
Amino acid	Phytase, U/kg	CWS[c]	CWC[d]	SCS[e]	Mean
His[g]					
	0	88.95	84.70	82.93	**85.52**
	500	89.65	85.33	82.95	**85.98**
	Mean	**89.30[x]**	**85.01[y]**	**82.94[z]**	
Lys[g,i]					
	0	86.73	81.58	84.00	**84.10**
	500	88.72	82.96	84.56	**85.41**
	Mean	**87.72[x]**	**82.27[z]**	**84.28[y]**	
Arg[g,i]					
	0	90.20	86.33	87.08	**87.87**
	500	91.30	87.36	87.60	**88.75**
	Mean	**90.75[x]**	**86.85[y]**	**87.34[y]**	
Total[g,i]					
	0	86.45	81.78	81.45	**83.22**
	500	87.83	82.85	82.38	**84.35**
	Mean	**87.14[x]**	**82.31[y]**	**81.91[y]**	
N[f,g]					
	0	81.65	73.99	74.96	**76.87**
	500	82.11	75.49	78.16	**78.59**
	Mean	**81.88[x]**	**74.74[z]**	**76.56[y]**	

[a]Each treatment mean represent 12 pigs.
[b]Diet type means within the same row that have different superscripts are different (P < .05)
[c]CWS = corn-wheat-soybean meal based diet.
[d]CWC= corn-wheat-canola meal based diet.
[e]SCS = sorghum-corn-soybean meal based diet.
[f]Phytase effect (P < .01)
[g]Diet type effect (P <.001)
[h]Phytase effect (P < .1)
[i]Phytase effect (P < .05)
[j]Phytase*Diet type effect (P < .01)
[k]Phytase*Diet type effect (P < .05)

.05). The influence of diet type on the AID of amino acids demonstrates the negative impact of canola meal on amino acid AID. This effect can partially be overcome by the addition of microbial phytase to the diet.

Apparent Ileal Digestibility of Calcium, Phosphorus, and Dry Matter. A trend ($P < .1$) towards an interaction of phytase and diet type was observed for Ca AID (Table 7). The addition of phytase resulted in an increase ($P < .01$) in the AID of Ca for all diet types, but the magnitude of this response was greatest for pigs fed the SCS based diet followed by pigs fed the CWC based diet and then by pigs fed the CWS based diet.

Phosphorus AID was improved ($P < .001$) by the addition of microbial phytase. On average, P AID improved 12.3 percentage units when microbial phytase was added to the diet. Microbial phytase addition also caused a small increase (1.1 percentage unit) in the AID of DM ($P < .05$).

Phosphorus and DM AID were both influenced ($P < .001$) by diet type. The AID of P and DM were highest for pigs fed the CWS based diet followed by pigs fed the SCS based diet and then by pigs fed the CWC based diet. These results confirm the negative impact of canola on AID as observed above for amino acids.

Table 7. The effects of microbial phytase addition to wheat-soybean meal, wheat-canola, or sorghum-soybean meal based diets on apparent ileal Ca, P and DM digestibility[a,b].

Item	Phytase, U/kg	Diet Type			Mean
		CWS[c]	CWC[d]	SCS[e]	
Ca[f,g,h]					
	0	32.71	13.44	9.78	**18.64**
	500	40.07	25.20	29.03	**31.43**
	Mean	**36.39[x]**	**19.32[y]**	**19.41[y]**	
P[f,g]					
	0	53.61	40.14	45.21	**46.32**
	500	66.27	52.41	57.23	**58.64**
	Mean	**59.94[x]**	**46.28[z]**	**51.22[y]**	
DM[g,I]					
	0	82.05	76.64	77.84	**78.85**
	500	82.88	77.46	79.39	**79.91**
	Mean	**82.47[x]**	**77.05[z]**	**78.61[y]**	

[a]Each treatment mean represent 12 pigs.
[b]Diet type means within the same row that have different superscripts are different (P < .05).
[c]CWS = corn-wheat-soybean meal based diet.
[d]CWC= corn-wheat-canola meal based diet.
[e]SCS = sorghum-corn-soybean meal based diet.
[f]Phytase effect (P < .001)
[g]Diet type effect (P < .001)
[h]Phytase*Diet type effect (P < .1)
[i]Phytase effect (P <.05)

Discussion

The addition of microbial phytase to pig diets has been shown to be a very effective method of increasing the P digestibility of plant based diets commonly fed to pigs, thereby decreasing the excretion of P. Phytase increases the digestibility of phytate P by hydrolyzingthe bond between the phosphate groups and the inositol ring of phytate. Each of the six phosphate groups of phytate can carry up to two negative charges at a neutral pH (Erdman, 1979). As a result, phytate has the ability to bind positively charged amino acids, peptides, or proteins (Cosgrove, 1980; Prattley et al., 1982; Anderson, 1985; Thompson, 1986) making them unavailable to the pig for absorption. Several studies have demonstrated that the addition of microbial phytase to swine diets will increase total tract N digestibility and the apparent ileal digestibility of many amino acids (Mroz et al., 1991; Khan and Cole, 1993; Kemme et al., 1995; Christensen and Nielson, 1995; Zhang and Kornegay, 1999; Johnston et al., 2000).

In the present study diet type had an effect on P digestibility, but there were no observed diet type by phytase interactions. Therefore, phytase had similar efficacies in all three diet types. On average, when 500 U of phytase per kilogram of diet were added, the ATTD of P was increased 9.45 percentage units. This 9.45 percentage unit increase represents a .38 g increase in P digestibility per kilogram of diet. If this value is divided by the P bioavailability of inorganic P sources (76.7%) as estimated by Kornegay et al. (1998) it can be concluded that 500 U of phytase per kilogram of diet can replace approximately .495 g of P from inorganic phosphate. This equivalency value is lower than most of those reported in the literature which all tend to be around 1 g of inorganic P for 500 U of phytase/kg of diet (Jongbloed et al., 1992; Kornegay et al., 1998; Radcliffe and Kornegay, 1998). There are several possible reasons for this. First, in this

study only one level of added phytase was used and no added levels of P were used. Therefore, calculations of equivalency values are crude at best. Second, the majority of the studies reported in the literature use corn-soybean meal based diets (Kornegay et al., 1998), and it is likely that equivalency values differ as the plant ingredients in the diet are changed. For example, four of the diets in this experiment contained wheat. Wheat provided a substantial amount of endogenous phytase activity to both the phytase supplemented and unsupplemented diets. Previous work has shown that the P ATTD response to phytase decreases for every U of added phytase activity (Kornegay et al., 1998). Therefore, due to the wheat phytase, pigs fed diets containing wheat had a higher starting position on the response curve, and therefore the response to added phytase would be expected to be less. Finally, pigs on this study were limit fed. Previous research has shown that pigs that are limit fed tend to be more efficient at digestion and absorption (NRC, 1998). Therefore, the P ATTD of pigs on this study fed diets without phytase may have been higher than would normally be observed on a commercial operation.

An interaction of diet type and phytase addition was observed for the ATTD of Ca. The addition of phytase to CWS and CWC based diets had little effect on the ATTD of Ca. However, the addition of microbial phytase to the SCS based diet resulted in a 13.6 percentage unit increase in the ATTD of Ca. The SCS based diet had the lowest analyzed level of Ca in the diet. Thus, it may have been the only diet that was marginally deficient in Ca, and was therefore the only diet where the ATTD of Ca was affected by phytase addition. In addition, the ATTD Ca of pigs fed the CWS based diet without phytase supplementation was high (69.61%), possibly due to the high endogenous phytase content of wheat.

In the present study, a phytase by diet interaction was observed for the AID of Val and Met. The addition of phytase to the CWS or CWC based diets improved the AID of Val and Met, but the addition of phytase to the SCS based diet resulted in a decrease in the AID of Val and Met. The AID of all other amino acids were improved with the addition of phytase, but this effect was not significant for Glu, Pro, Cys, Ile, Leu and His. Changes in the AID of amino acids ranged from .76 to 2.21 percentage units for the CWS based diet, from .39 to 2.3 percentage units for the CWC based diet, and from -1.68 to 2.67 percentage units for the SCS based diet (Table 8). On average, amino acid AID was improved 1.36, 1.18, and .78 percentage units, respectively for CWS, CWC, and SCS based diets. For the experiment reported in Chapter IV, amino acid AID changed from -1.97 to 4.53 percentage units when 500 U of phytase were added per kilogram of diet. Only His had a numerically lower AID when phytase was added to the diet. If His is excluded, then on average 500 U of phytase per kilogram of diet improved amino acid AID an average of 2.3 percentage units.

Improvements in the AID of amino acids reported as a percentage unit increase are not comparable between different diet types because the amino acid profiles of the diets are different. Therefore, Table 9 shows the amount of each amino acid that could be replaced in the diet when 500 U of phytase are added per kilogram of diet. Values from this study were calculated by multiplying the percentage unit improvement in amino acid digestibility by the amount of each amino acid in the diet. Values shown from Chapter IV were determined by setting the phytase response equation equal to the amino acid response equation and solving for 500 U of phytase per kilogram of diet. The values from Chapter IV are therefore adjusted based on the

Table 8. Percentage unit improvements in apparent ileal digestibilities of amino acids when phytase was added to the diet at a level of 500 U per kilogram of diet.

Item	Diet				
	CWS[a]	CWC[b]	SCS[c]	Mean	Chapter IV[d]
	----------Percentage unit improvement----------				
Total AA	1.38	1.07	.93	1.13	3.01
N	.46	1.50	3.2	1.72	1.49
Asp	2.21	2.3	1.25	1.92	3.31
Thr	2.04	1.44	2.12	1.96	3.33
Ser	1.64	.64	2.20	1.52	2.54
Glu	.82	.65	.58	.68	2.77
Pro	1.00	.39	2.67	1.34	.59
Gly	1.87	2.20	1.24	1.77	4.53
Ala	2.01	1.21	.66	1.30	1.86
Cys	.77	1.70	-.81	.56	2.24
Val	.76	1.30	-1.68	.12	2.06
Met	1.88	.71	-.88	.57	1.96
Ile	.86	1.11	-.97	.33	1.80
Leu	1.14	.62	.26	.67	1.26
Tyr	1.73	1.03	1.59	1.45	2.34
Phe	1.24	1.58	.47	1.10	2.26
His	1.00	.63	.02	.45	-1.97
Lys	1.99	1.38	.56	1.31	1.63
Arg	1.10	1.03	.52	.88	2.40

[a]CWS = corn-wheat-soybean meal based diet.
[b]CWC= corn-wheat-canola meal based diet.
[c]SCS = sorghum-corn-soybean meal based diet.
[d]Estimated improvement in the apparent ileal digestibility of amino acids in Chapter IV when 500 U of phytase were added per kilogram of diet. Estimates were calculated by setting apparent ileal digestibility response equations for CP and phytase equal to each other and solving for 500 U of phytase per kilogram of diet.

Table 9. Amino acid replacement values of 500 U of phytase per kilogram of diet.

	Diet				
Item	CWS[a]	CWC[b]	SCS[c]	Mean	Chapter IV[d]
	----------Replacement value, % of diet----------				
Total AA	0.174	0.133	0.124	0.145	0.334
N	0.010	0.032	0.078	0.038	0.024
Asp	0.024	0.020	0.015	0.020	0.034
Thr	0.010	0.007	0.011	0.009	0.014
Ser	0.010	0.003	0.014	0.009	0.012
Glu	0.024	0.019	0.016	0.019	0.055
Pro	0.010	0.004	0.025	0.013	0.004
Gly	0.010	0.013	0.006	0.010	0.020
Ala	0.013	0.008	0.006	0.010	0.012
Cys	0.002	0.006	-0.002	0.002	0.005
Val	0.004	0.008	-0.010	0.001	0.011
Met	0.005	0.002	-0.002	0.002	0.004
Ile	0.004	0.005	-0.005	0.002	0.008
Leu	0.012	0.006	0.004	0.008	0.013
Tyr	0.007	0.004	0.008	0.006	0.008
Phe	0.008	0.009	0.003	0.007	0.012
His	0.003	0.002	0.000	0.002	-0.006
Lys	0.013	0.009	0.003	0.008	0.009
Arg	0.008	0.007	0.004	0.006	0.016

[a]CWS = corn-wheat-soybean meal based diet.

[b]CWC= corn-wheat-canola meal based diet.

[c]SCS = sorghum-corn-soybean meal based diet.

[d]Estimated improvement in the apparent ileal digestibility of amino acids in Chapter IV when 500 U of phytase were added per kilogram of diet. Estimates were calculated by setting apparent ileal digestibility response equations for CP and phytase equal to each other and solving for 500 U of phytase per kilogram of diet.

bioavailability of each amino acid from the diet, whereas the values calculated from this study are not. Based on these results, 500 U of phytase per kilogram of diet can replace .002 to .024 percentage unit of individual amino acids in the CWS based diets, from .002 to .020 percentage unit of individual amino acids in the CWC based diets, and from -.010 to .020 percentage unit of individual amino acids in the SCS based diets. Averages were .010, .008, and .006 percentage unit, respectively for CWS, CWC, and SCS based diets. In Chapter IV, the addition of 500 U of phytase per kilogram of diet could replace -.006 to .055 percentage unit of amino acids, with the average being .014 percentage unit. To compare the effects of phytase in various diet types, it is first important to investigate the AID of amino acids in the diet when no phytase is added. All amino acids assayed followed the same pattern of digestibility in this study. Pigs fed the CWS based diets had the highest AID of all amino acids, followed by pigs fed the SCS based diet, and then by pigs fed the CWC based diet (Table 10). The endogenous phytase activity present in wheat is most likely the primary reason for the higher AID of amino acids in the CWS based diet. Likewise, the high phytate content of canola is most likely the primary reason for the relatively low AID of amino acids in the CWC based diet. The digestibility of amino acids in all diet types was higher in this study compared to those reported in Chapter IV in which all diets were corn-soybean meal based. The lack of endogenous phytase activity combined with the low level of dietary protein maybe responsible for the low AID of amino acids reported in Chapter IV. The AID coefficients would be considerably higher if they were adjusted for endogenous N losses.

Theoretically, in diets with lower AID for amino acids there is more potential room for improving amino acid digestibilities when phytase is added to the diet. Based on that logic, the

Table 10. Apparent ileal digestibilities of amino acids in each diet type without the addition of microbial phytase.

Item	Diet			
	CWS[a]	CWC[b]	SCS[c]	Chapter IV[d]
	----------%----------			
Total AA	86.45	81.78	81.45	77.3
N	81.65	73.99	74.96	66.7
Asp	82.96	74.22	80.84	74.4
Thr	78.44	71.96	73.87	66.0
Ser	85.65	79.65	80.54	78.0
Glu	91.66	89.32	85.47	82.3
Pro	88.21	82.74	74.97	78.9
Gly	78.79	73.43	70.96	62.9
Ala	80.64	76.56	80.27	71.4
Cys	87.06	83.10	78.25	74.7
Val	83.84	78.26	80.53	70.0
Met	88.17	86.55	84.05	75.8
Ile	84.38	77.96	81.19	73.0
Leu	86.99	82.94	83.81	79.9
Tyr	83.94	77.60	80.21	73.8
Phe	86.92	82.53	83.37	78.6
His	88.95	84.70	82.93	80.4
Lys	86.73	81.58	84.00	72.7
Arg	90.20	86.33	87.08	84.0

[a]CWS = corn-wheat-soybean meal based diet.
[b]CWC= corn-wheat-canola meal based diet.
[c]SCS = sorghum-corn-soybean meal based diet.
[d]Observed apparent ileal digestibility values for pigs fed Diet 3 in Chapter IV, which was formulated to contain 10.2% CP.

largest changes in amino acid AID should have been seen in pigs fed the CWC based diet. However, the largest improvements when 500 U of phytase were added per kilogram of diet were observed in pigs fed the CWS based diet, and the smallest improvements were seen in the SCS based diets. The addition of 500 U of phytase per kilogram of diet improved the total amino acid AID by 1.38, 1.07, and .93 percentage units for pigs fed CWS, CWC, and SCS based diets, respectively. By multiplying the improvement in total amino acid AID by the amino acid content of each diet, it was determined that 500 U of phytase per kilogram of diet could replace .174, .133, and .124 percentage units of amino acids, respectively for CWS, CWC, and SCS based diets. Pigs fed the SCS based diet had lower AID of all amino acids, compared with pigs fed the CWS based diets, yet the addition of phytase to the CWS based diets had a greater effect than the addition of phytase to the SCS based diet. Therefore, it seems reasonable to conclude that there are other anti-nutritive factors in sorghum that are causing this lower AID of amino acids. The same appears to be true for the CWC based diet. Canola meal is high in phytate. Therefore, this diet had the highest concentration of substrate for the phytase enzyme. However, the efficacy of phytase in this diet was lower than it was for the CWS based diet. Therefore, there may be other antinutritive factors in canola meal in addition to phytate, which are contributing to the lower amino acid AID.

Several studies have reported increases in amino acid or N digestibility when phytase was added to the diet (Mroz et al., 1991; Khan and Cole, 1993; Kemme et al., 1995; Christensen and Nielson, 1995; Zhang and Kornegay, 1999; Johnston et al., 2000). Improvements in the digestibility of amino acids when phytase is added to the diet reported in the literature have ranged from 3.5% (Mroz et al., 1991) to 12.8% (Officer and Batterhan, 1992; Khan and Cole;

1993). Recently, Zhang and Kornegay (1999) reported that 500 U of phytase per kilogram of diet caused an increase in the AID of amino acids in the range of 3.39 to 10.72%. In the present study, improvements in the AID of amino acids improved from .47 to 3.56%. Diet type had a profound affect on amino acid AID, and there were few interactions between diet type and phytase efficacy.

Implications

The addition of microbial phytase to CWC, CWC, or SCS based diets fed to swine improves the apparent ileal digestibilities on amino acids, P and Ca. Diet type also affected the digestibility of amino acids, Ca and P, but few interactions of diet type and phytase were observed.

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Chapter VII

The Effects of Hemicell on Mineral, Energy and Amino Acid Digestibility in Finishing Pigs Fitted with Steered Ileo-Cecal Valve Cannulas

ABSTRACT. Twelve crossbred barrows (BW = 44 kg), fitted with steered ileo-cecal valve (SICV) cannulas, were used to investigate the effects of Hemicell (β-mannanase) on the apparent total tract digestibilities (ATTD) of energy, Ca, P, DM, and the apparent ileal digestibilities (AID) of Ca, P, DM and amino acids. Pigs were randomly allotted to four dietary treatments in three 4 x 4 Latin squares. All diets were corn-soybean meal-based. Diets 1 and 2 contained 12% CP, and diets 3 and 4 contained 16% CP. Diets 2 and 4 were supplemented with Hemicell at a level of .05% of the diet. Calcium and P levels were .54% and .45% for the low CP diets and .53% and .45% for the high CP diets, respectively. Pigs were individually housed in metabolism pens (1.2m x 1.2m). Water was supplied *ad libitum*, and feed was given at a level of 9% of metabolic BW ($BW^{.75}$) per day in two daily feedings. Each of four 2-wk periods consisted of a 7-d adjustment, a 3-d total collection, a 12-h ileal digesta collection, a 3-d readjustment, and a second 12-h ileal digesta collection. There were no interactive effects of Hemicell and CP level; therefore, only main effects are reported. Increasing the level of CP in the diet from 12 to 16% improved ($P < .001$), ADG, G:F, and the AID of DM, Ca, P, N and all amino acids measured. In addition, the ATTD of P ($P < .01$) was increased by feeding this high CP diet, but there was no effect ($P > .1$) on the ATTD of Ca, energy, or DM. The addition of Hemicell to the diet increased the AID of DM ($P < .001$) and the ATTD of energy ($P < .05$). Although, numerical means favored Hemicell, differences were not significant ($P > .10$) for the AID of Ca, P, and amino acids or the ATTD of Ca, P, and DM. Based on the results of this study, Hemicell may be

a useful feed additive for increasing the energy availability in corn-soybean meal-based diets fed to pigs.

Key words: Pigs, Digestibility, β-mannanase, Minerals, Energy, Amino Acids

Introduction

An increased awareness of the potential environmental impact of excess nutrients in swine manure has caused swine nutritionists to focus on anti-nutritional factors in the diet. Hence, interest has grown in the addition of feed additives to the diet which decrease the detrimental effects of these anti-nutritional factors. One such group of anti-nutritional factors are β-mannans, which consist of a backbone of β-1,4 linked mannose sugars and β-1,6 linked galactose or glucose side chains. Interest in the anti-nutritional effects of these compounds originated with the addition of guar gum to poultry diets. It was found that the addition of guar gum to poultry diets decreased gastric emptying, slowed gastrointestinal transit time, increased the water holding capacity of the diet, and decreased protein retention, all of which led to a decreased performance of birds fed diets including guar gum (Couch et al., 1967). These detrimental effects of feeding guar gum were attributed to a high level of trypsin inhibitor activity in guar gum, and to a high galactomannan content. The detrimental effects of these compounds are primarily associated with their high water holding capacity. The addition of Hemicell (ChemGen Corporation, Gaithersburg, MD; U.S. patent #5429828), a β-mannanase, to swine diets containing corn, soybean meal and barley hulls led to increased Ca and energy digestibility (Anonymous, 1999). Pigs in the United States are typically fed corn-soybean meal based diets which contain very low levels of β-mannans. However, energy and protein are the two most costly nutrients added to the diet. Therefore, if the addition of β-mannanase to the diet could improve energy or protein digestibility, it may be economically feasible to add this product to swine diets. To investigate this possibility, this study was designed to test the effects of adding β-mannanase to swine diets on amino acid, energy, and mineral digestibilities.

Materials and Methods

Animals, Housing, and Analysis. Twelve crossbred barrows fitted with steered ileo-cecal valve (SICV) cannulas (described in Chapter III) were used in three replicates of a 4x4 Latin square (Table 1) to test the effects of added Hemicell on mineral, energy and amino acid digestibilities. All diets were corn-soybean meal-based (Table 2). Diets 1 and 2 contained 12% CP, and diets 3 and 4 contained 16% CP. Diets 2 and 4 were supplemented with Hemicell at a level of .05% of the diet. Calcium and P levels were .54% and .45% for the low CP diets and .53% and .45% for the high CP diets, respectively. All diets contained .05% Cr_2O_3 as an indigestible marker for calculation of apparent digestibility coefficients. Pigs were individually housed in metabolism pens (1.2 m^2). Water was supplied *ad libitum* and feed was supplied at a level of 9% of metabolic BW ($BW^{.75}$) per d. The average starting BW was 44 kg and the average ending BW was 75 kg. Pigs were fed twice daily (0800 h and 1800 h).

Each of the four 2-wk periods consisted of a 7-d adjustment followed by a 3-d total collection, a 12-h ileal digesta collection, a 3-d readjustment, and a second 12-h ileal digesta collection. During the 12-h ileal collection, digesta was emptied from the collection bags and placed on dry ice as soon as it appeared. It was then weighed and placed in an ultra low temperature freezer (-80 ^oC) every hour. Feces and urine were collected separately during each 3-d total collection period. Feces were collected by placing a plastic bag over the anus of each pig following the procedures of van Kleef et al. (1994). Urine was collected in buckets from drop pans under each pen. Twenty-five ml of a 25% HCl solution were added to each bucket, twice daily (when the bucket was emptied), to lower urine pH and minimize N volatilization.

Table 1. Experimental design.

		Latin square no.											
		1				2				3			
Period	Pen	1	2	3	4	5	6	7	8	9	10	11	12
1		1[a]	2	3	4	1	2	3	4	1	2	3	4
2		2	1	4	3	3	4	1	2	4	3	2	1
3		3	4	1	2	4	3	2	1	2	1	4	3
4		4	3	2	1	2	1	4	3	3	4	1	2

[a]Dietary treatments.

Table 2. Composition of basal diets.

Item	Diet	
	High protein[a]	Low protein[b]
Ingredient composition, %	----------%----------	
Corn	75.85	86.96
Soybean Meal (44%)	21.71	10.47
Defluorinated phosphate	.26	.49
Vit. Premix[c]	.20	.20
TM premix[d]	.05	.05
Se premix[e]	.05	.05
Limestone	.88	.78
Salt	.30	.30
Cr-premix[f]	.20	.20
Corn starch[g]	.50	.50
Calculated analysis		
CP, %	16.0	12.0
Lysine, %	.82	.52
ME (kcal/kg)	3363	3318
P, %	.40	.40
Ca, %	.50	.50

[a]Used to make diets 3 and 4.
[b]Used to make diets 1 and 2.
[c]Supplied per kilogram of diet: 4400 IU of Vitamin A, 440 IU of Vitamin D_3, 11 IU of Vitamin E, 2.2 mg of Vitamin K, 4.4 mg of riboflavin, 22 mg of calcium pantothenate, 22 mg of niacin, 0.022 mg of vitamin B_{12}, 440 mg of choline chloride, 0.44 mg of biotin, 3.9 mg of folic acid, 10 mg of thiamin•HCl, 3.9 mg of pyridoxine•HCl, 82.5 mg of ethoxyquin and 3.6 mg of virginiamycin.
[d]Supplied per kilogram of diet: 44 mg of manganese, 47.5 mg of zinc, 50 mg of iron, 6.25 mg of copper, and 2 mg of iodine.
[e]Supplied 0.3 mg selenium per kilogram of diet.
[f]Cr-premix consisted of 1 part Cr_2O_3 to 3 parts starch on a wt:wt basis.
[g]Corn starch was replaced by phytase Hemicell in diets 2 and 4.

Urine and feces were collected twice per day during the collection periods and frozen at -20°C for subsequent analysis. Pigs were weighed every 2 wk at the end of each period, at which time feeding levels were adjusted.

Fecal samples were dried at 60°C to a constant weight in a forced air oven and ileal samples were freeze dried. Diet, fecal, and ileal digesta samples were analyzed for Ca, P, and Cr following nitric-perchloric acid (5:3, vol:vol) wet digestion. Total P concentrations were assayed photometrically using the vandomolybdate procedure (AOAC, 1990) and Ca and Cr concentrations were determined by atomic absorption spectrophotometry following the manufacturer's recommended procedures (model 5100 PC, Perkin Elmer, Norwalk, CT). Samples were analyzed for N content using the Kjeldahl procedure (AOAC, 1990). Diets and fecals were analyzed for energy content using a bomb calorimeter (PARR 1271 Bomb Calorimeter, PARR instrument Company, Moline, IL). Diets and ileal digesta were hydrolyzed in 6 N HCl for 24 h and analyzed for amino acid content using HPLC by the Experiment Station Chemical Laboratory at the University of Missouri-Columbia (Columbia, MO 65211).

Description of Cannula. The cannula was described in Chapter III. Briefly, the SICV cannula consists of 5 parts; an inner cannula barrel (barrel, 120 mm length, 25 mm i.d.; 32 mm o.d.; flange, 70 mm o.d.), an outer cannula barrel (barrel, 43 mm length, 33 mm i.d., 41 mm o.d.; flange, 80 mm o.d.), an internal ring (2.0 mm thick and 35.0 mm i.d.) attached to a nylon cord, an external ring (2.0 mm thick and 34 mm o.d.), and a cylindrical stopper (24 mm o.d. and 60 mm length).

Surgical Procedures. Surgical insertion of the SICV cannula was as described in Chapter III. Briefly, the average BW of the pigs at the time of surgery was 40 kg. Feed was withdrawn from pigs 36 h prior to surgery and water was removed 12 h prior to surgery. Anasthesia was

induced with an i.m. injection of medetomidine (80 μg/kg BW), ketamine (10 mg/kg BW), and butorphanol (0.2 mg/kg BW). Pigs were maintained on halothane in oxygen *via* an endotracheal tube for the duration of the surgical procedure. Prior to surgery pigs were given an i.v. injection of Naxcel (0.45 mg/kg BW) and Banamine (0.23 mg/kg BW). On the day following surgery pigs were fed 50 g of diet, which was increased 100 g/d (split into 2 daily feedings, 0800 and 1800) up to a level of 9% $BW^{.75}$. Based on this feeding regimen, a 40 kg pig would be at the intended feeding level approximately 12 d post-surgery.

Statistical Analysis. Data were analyzed using the GLM procedures of SAS (1990). Pen within period was the experimental unit for fecal data, and the model included square, period, treatment, and carry-over effect. Collection period of each pig was the experimental unit for ileal data, and the model included square, period, treatment, and carry-over effect. Contrast statements compared protein levels, Hemicell addition, and the interaction of protein and Hemicell.

Results

Dietary Composition. The analyzed dietary composition (Table 3) was in good agreement with the calculated dietary composition (Table 2). Analyzed CP levels were 12.55% for the low CP diets and 16.6% for the high CP diets. The average starting and ending BW were 44 and 75 kg, respectively. Based on the NRC (1998) for this weight range, the protein requirement is 15.5%. This protein requirement decreases to 13.2% (0.60% lysine) at 80 kg BW. Therefore, the low CP diet was protein deficient for pigs throughout the study, while the high CP

Table. 3. Analyzed dietary composition.

Item	Diets			
Crude protein, %[a]	12	12	16	16
Hemicell, %[a]	0	0.05	0	0.05
DM, %	88.56	88.94	89.13	89.38
CP, %	12.62	12.47	16.60	16.60
N, %	1.990	2.035	2.655	2.705
Ca, %	0.544	0.544	0.526	0.526
Cr, %	0.029	0.029	0.027	0.027
P, %	0.451	0.451	0.448	0.448
Energy (kcal/kg)	3952	3952	3982	3982
Asparagine, %	1.070	1.060	1.595	1.595
Threonine, %	0.460	0.455	0.615	0.615
Serine, %	0.555	0.535	0.705	0.700
Glutamine, %	2.345	2.320	3.090	3.080
Proline, %	0.905	0.885	1.075	1.075
Glycine, %	0.520	0.515	0.710	0.705
Alanine, %	0.805	0.795	0.945	0.950
Cysteine, %	0.270	0.265	0.320	0.315
Valine, %	0.640	0.640	0.845	0.855
Methionine, %	0.235	0.235	0.295	0.285
Isoleucine, %	0.490	0.495	0.700	0.710
Leucine, %	1.330	1.310	1.585	1.580
Tyrosine, %	0.430	0.415	0.555	0.565
Phenylalanine, %	0.635	0.625	0.850	0.850
Histidine, %	0.365	0.365	0.480	0.485
Lysine, %	0.570	0.560	0.880	0.880
Arginine, %	0.775	0.770	1.125	1.130

[a]Calculated composition.

diet was more than adequate. Gross energy content of the diets was 3952 kcal/kg and 3982 kcal/kg for the low and high protein diets, respectively. The estimated metabolizable energy requirement for pigs from 50 to 80 kg is 8,410 kcal ME/d and increases to 10,030 kcal ME/d from 80-120 kg BW. If the daily ME intake of pigs in this study is calculated based on a daily intake of 9% of metabolic BW and an estimated ME concentration in the diet of 3455 kcal/kg, the pigs were consuming 5,312 kcal ME/d at the start of the study and 7,925 kcal ME/d by the end of the study. Therefore, all diets were energy deficient for the duration of the study. Calcium and P levels were .544% and .451%, respectively, for the low CP diets and .526% and .448%, respectively, for the high CP diets. These levels are similar to the NRC (1998) recommendations of 0.50 and 0.45 % Ca and P, respectively for pigs weighing 50 to 80 kg. Thus, the Ca:P ratios of 1.2:1 and 1.17:1 for the low and high CP diets, respectively, were in agreement with the NRC (1998) recommendation of 1.1:1 to 1.25:1.

Performance. Pigs fed the high CP diets grew faster ($P < .001$) than those fed the low CP (Table 4; $P < .001$). The net result was that pigs fed the higher CP diet grew an additional 122 g/d, demonstrating that the low CP diet was indeed deficient in protein. This improved growth rate was due to an improved gain:feed ratio (Table 4) which increased from .339 in the low CP diets to .420 in the high CP diets. There were no effects of Hemicell addition or any Hemicell by protein level interactions on growth performance. This was probably influenced by the fact that the length of each period was only 2 wk.

Total Tract Digestibilities. There were no interactive effects of Hemicell supplementation and CP level, so only the main effects of each will be presented. Increasing the level of CP in the diet from 12 to 16% increased the digestibility of P ($P < .01$) while having no effect ($P > 0.1$) on Ca, energy or DM digestibility (Table 5). Phosphorus digestibility improved

Table 4. The effects of crude protein level and Hemicell supplementation on ADG and feed conversion.

Item	Diets[a,b]								
Crude protein, %[c]	12	12	16	16			Probability		
Hemicell, %[c]	0	0.05	0	0.05	SEM	C.V.	CP	HEM	CP*HEM
ADG, g/d	501.6	507.1	644.1	605.9	26.22	16.09	.001	.50	.23
Gain:Feed, g/kg	335	343	436	404	19	17.28	.001	.48	.16

[a]Each mean represents 12 observations except digesta which represents 24 observations.
[b]Pigs were fed at a level of 9% of their metabolic BW ($BW^{.75}$).
[c]Calculated composition.

Table 5. Apparent total tract digestibility of dry matter, energy, Ca and P and N balance of finishing pigs fed low and adequate protein diets with and without Hemicell.

Item	Diets[a,b]								
Crude protein, %[c]	12	12	16	16			Probability values		
Hemicell, %[c]	0	0.05	0	0.05	SEM	C.V.	CP	Hem	CP*HEM
Apparent total tract digestibilities, %									
Dry matter	89.80	89.97	90.00	90.30	.20	0.8	.30	.20	.92
Energy	88.77	89.04	89.00	89.54	.21	0.8	.15	.04	.59
Ca	50.65	52.37	51.14	49.91	1.44	9.7	.66	.72	.19
P	36.41	35.75	39.34	39.94	1.04	9.5	.01	.89	.38
N balance									
N intake, g	30.24	30.13	39.77	40.20	.31	2.9	.001	.59	.36
Fecal N, g	3.79	3.62	4.07	3.92	.10	9.4	.01	.14	.94
Urinary N, g	10.37	10.64	10.76	10.99	.89	28.9	.68	.78	.98
N retained, g	16.08	15.86	24.94	25.29	.91	15.4	.001	.94	.76
N digestibility, %	87.42	87.89	89.66	90.14	.29	1.1	.001	.11	.98
N retention, %	52.91	52.00	62.75	62.62	2.43	14.6	.001	.83	.87

[a]Each mean represents 12 observations.
[b]Pigs were fed at a level of 9% of their metabolic BW ($BW^{.75}$).
[c]Calculated composition.

approximately 3.5 percentage units as the CP level in the diet was increased from 12 to 16%, and was not affected by Hemicell supplementation. Pigs fed the higher CP diets consumed more N and had higher N digestibilities ($P < .001$) and retention (Table 5; $P < .001$). In addition, fecal N excretion was increased ($P < .01$), but no change was seen in urinary N excretion (Table 5; $P > .1$).

The addition of Hemicell to the low or high CP based diets resulted in an increased energy digestibility ($P < .04$) of approximately 0.41 percentage units (Table 5). The addition of Hemicell, however, had no effect ($P > .1$) on Ca, P or DM digestibility (Table 5). While Hemicell addition did not significantly affect ($P > .1$) any of the N parameters measured, there was a slight numerical improvement ($P = .11$) in N digestibility resulting from a numerical decrease in fecal N excretion ($P = .14$) when Hemicell was supplemented to the diet.

Apparent Ileal Digestibilities. As with total tract measurements, there were no interactive effects of Hemicell supplementation and CP level, so only the main effects of each will be presented. Increasing the level of CP in the diet from 12 to 16% resulted in improvements ($P < .001$) in the digestibilities of DM, Ca, P, N, and all amino acids measured (Table 6). Dry matter, Ca, P, and N digestibilities were improved 1.32, 5.38, 7.19, and 7.33 percentage units, respectively as the level of CP in the diet increased from 12 to 16%. Amino acid digestibilities were increased from 2.6 to 10.5 percentage units as protein level in the diet increased.

The addition of Hemicell resulted in an improvement in DM digestibility (Table 6; $P < .001$). With Hemicell addition, DM digestibility was improved 1.6 percentage units which may have been due to an increased carbohydrate digestibility as indicated by the improved energy digestibility seen in Table 5. No significant improvements ($P > .1$) were seen in the

Table 6. Ileal digestibility of dry matter, energy, Ca, P, N and amino acids of finishing pigs fed low and adequate protein diets with and without Hemicell.

Item, %	Diets[a,b]								
Crude protein, %[c]	12	12	16	16			Probability values		
Hemicell, %[c]	0	0.05	0	0.05	SEM	C.V.	CP	HEM	CPxHEM
Dry matter	74.13	75.32	75.05	77.04	.37	2.4	.001	.001	.80
Ca	51.52	53.47	56.61	59.14	1.31	11.6	.001	.12	.87
P	41.22	41.87	48.19	49.27	1.02	11.1	.001	.61	.87
N	70.61	71.07	77.48	78.87	.58	3.8	.001	.14	.75
Total amino acids	77.34	77.69	82.25	83.3	.46	2.8	.001	.20	.84
Aspartic acid	74.16	74.25	80.75	82.02	.54	3.4	.001	.26	.69
Threonine	65.01	65.13	72.51	74.24	.78	5.5	.001	.26	.67
Serine	76.59	76.64	80.74	81.77	.49	3.0	.001	.32	.70
Glutamine	83.48	83.56	86.83	87.53	.36	2.1	.001	.33	.78
Proline	72.77	70.39	81.20	81.28	1.39	8.9	.001	.31	.41
Glycine	64.09	63.53	73.37	75.33	.78	5.5	.001	.53	.31
Alanine	74.98	75.08	78.06	79.63	.58	3.7	.001	.19	.56
Cystine	75.33	75.59	78.40	79.41	.56	3.6	.001	.25	.95
Methionine	79.64	79.98	83.01	84.35	.55	3.3	.001	.16	.73
Valine	75.02	75.42	79.81	81.17	.54	3.4	.001	.14	.84
Isoleucine	74.57	74.68	80.79	82.03	.52	3.3	.001	.26	.72
Leucine	81.60	81.93	83.95	84.85	.42	2.5	.001	.19	.95
Thyrosine	75.80	75.57	80.76	82.04	.57	3.6	.001	.37	.46
Phenylalanine	80.45	80.98	84.38	85.32	.44	2.6	.001	.16	.88
Histidine	81.95	82.23	85.81	86.74	.38	2.2	.001	.13	.82
Lysine	74.15	74.05	82.47	83.75	.56	3.5	.001	.31	.56
Arginine	84.78	84.95	89.06	89.71	.30	1.7	.001	.29	.83

[a]Each mean represents 24 observations.

[b]Pigs were fed at a level of 9% of their metabolic BW ($BW^{.75}$).

[c]Calculated composition.

digestibilities of Ca, P, N, or any of the amino acids measured (Table 6). However, a numerical improvement was seen for Ca digestibility (P = .12) and N digestibility (P = .14). This numerical improvement in N digestibility follows the same pattern seen in total tract N digestibility. Although not statistically significant, the numerical means of ileal digestibilities of all amino acids were increased (P < 0.13 to 0.33), except for the digestibilities of proline, glycine, tyrosine, and lysine for the 12% CP diet.

Discussion

Nutritionists aim to maximize the utilization of dietary nutrients and lean tissue accretion in growing-finishing pigs. The pig is not physiologically capable of utilizing 100% of the nutrients in the diet. However, by adding enzymes to the diet which degrade anti-nutritional compounds in the feed, it is possible to increase the digestibility of nutrients in the diet. The enzyme Hemicell, is a β-mannanase, which hydrolyzes the β-1,4-linkages between mannose sugar units in β-mannans. In a study by the Taiwanese Sugar Company (Anonymous, 1999), it was shown that the addition of Hemicell to a corn-soybean meal based diet containing .357% barley hulls, resulted in increased energy digestibility in grower pigs. In the present study the effects of Hemicell addition to corn-soybean meal based grower-finisher diets was investigated. Corn contains only trace amounts (~.09%) of β-mannan, and soybean meal contains between 1.2 (48% SBM) and 1.48 % (44% SBM) β-mannan. Two different protein levels were fed in this study to investigate the effects of substrate concentration on enzyme effects. Based on these estimates of the β-mannan content of corn and soybean meal, the 12% CP diet contained approximately .112% β-mannan, and the 16% CP diet contained approximately .388% β-mannan.

Very few studies have investigated the use of β-mannanase in pig diets. Interest in galactomannans in pig diets originally stemmed from the use of pigs as a model for humans to test for dietary factors which could aid in the treatment of non-insulin dependent diabetics. Rainbird et al. (1984), using pigs fitted with two re-entrant cannulas placed in the jejunum, found that the inclusion of guar gum (6.7 g/L) in a glucose (20 g/L) or maltose (20 g/L) infusate decreased glucose and water absorption from the jejunum. They also found that endogenous N secretion into the jejunal lumen was increased. In more recent work at that laboratory, Rainbird and Low (1986a,b) reported delayed gastric emptying, increased gastric pH, and increased digesta viscosity when guar gum was included in the diet. Interest in adding a β-mannanase to the diet increased as a result of the findings of several studies (Anderson and Warnick, 1964; Vohra and Kratzer, 1965; Ray et al., 1982) in chicks where a decrease in the detrimental effects of guar gum was observed when a crude or commercial preparation of hemicellulase was added to the diet. In 1982, Ray et al. isolated a mannanase from a commercially available hemicellulase preparation. By adding this enzyme to the diet of chicks containing 2% guar gum, they were able to eliminate the detrimental effects of guar gum compared with the positive control.

Pettey et al. (1999) reported on two studies in nursery pigs where Hemicell was included in the diet at a level of .05%. In both studies they found an improvement in ADG and feed efficiency with Hemicell addition. In the second experiment, pigs fed the diet supplemented with .05% Hemicell had similar ADG and feed efficiency to those fed a diet supplemented with soybean oil to provide an additional 100 Kcal ME/kg of diet. More recently, Pettey et al. (2000) reported on a growing-finishing trial and a metabolism trial investigating the effects of Hemicell addition to a corn-soybean meal based diet fed to growing-finishing pigs. They found an improved ADG and feed efficiency in pigs fed diets supplemented with .05% Hemicell.

However, in the balance trial they found no effect on Hemicell on energy, N, P, or dry matter digestibility.

In 1999, the Taiwan Sugar Company (Anonymous, 1999), using the commercial β-mannanase preparation, Hemicell, at an inclusion rate of .5 kg per ton to swine diets, observed an increased energy and Ca digestibility compared with unsupplemented animals. They also observed a decreased fecal DM excretion from pigs fed diets supplemented with Hemicell.

In the present study, the addition of Hemicell at a rate of .05% of the diet resulted in increased energy ATTD and DM AID. This is consistent with the findings of Taiwan Sugar Compay (Anonymous, 1999). Improved energy ATTD was also observed in their study. In addition, the increase in DM ATTD in this study is in agreement with their finding of a decreased fecal DM excretion. However, in the present study, differences observed in ileal DM digestibility were not observed for DM ATTD. This discrepancy between DM AID and ATTD may be due to energy absorption that occurs in the large intestine. Microorganisms in the large intestine are capable of utilizing substrates and converting them to volatile fatty acids (VFA). These VFA can be absorbed in the large intestine and utilized as energy sources for various metabolic processes. It seems reasonable to deduce from this study that the pig may be adjusting to the detrimental effects of β-mannans in the small intestine by increasing absorption in the large intestine. This adjustment, however, is not fully adequate to overcome the detrimental effects of β-mannans on the ATTD of energy. By adding β-mannanase to the high CP diet, the ATTD of energy was increased from 89.00 to 89.54%. This increase, while small, is important because of the cost of adding ingredients high in energy to the diet.

One of the most interesting observations in this study was the numerical increase in amino acid digestibilities when Hemicell was added to the diet. None of these increases were

statistically significant. However, with the exception of proline, tyrosine, and lysine, all amino acids measured were numerically increased by the addition of Hemicell. Probability values ranged from .13 to .33, except for glycine which was .53. β-mannans in the diet can decrease amino acid digestibilities by decreasing amino acid absorption and by increasing endogenous protein loss by increasing epithelial cell sloughage in the small intestine. The results of this study suggest that the addition of Hemicell to the diet may counteract some of these effects. The absence of a significant effect of Hemicell on amino acid digestibilities may have been related to the small number of animals used in this study, the shortness of time on each diet, the CP levels fed, and the relatively small amount of dietary substrate on which β-mannanase could act.

Implications

Based on the results of this study supplementing pig diets with Hemicell improves energy ATTD and DM AID. Therefore, diets formulated for pigs that include Hemicell may be formulated to a moderately lower energy content and produce similar performance. In addition, it appears that Hemicell, may also improve the AID of some amino acids and decrease endogenous N losses. Further research needs to be conducted to confirm these findings.

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Chapter VIII

General Discussion

The number of swine producers in the United States has been decreasing while the number of animals per farm has been steadily increasing. As a result of increasing animal densities per farm, manure production per farm has also increased. Historically, the primary goal of the nutritionist was to formulate a diet that optimized ADG at the lowest cost per unit of gain. As packers began to offer premiums based on carcass composition, nutritionists had to focus on lean tissue gain as opposed to total ADG. Now, with increasing emphasis on minimizing nutrient concentrations in the waste, nutritionists are searching for methods to minimize nutrient excretion without sacrificing performance or increasing the cost of the diet.

One of the easiest ways to decrease nutrient excretion has been to avoid over formulation of swine diets. Several feeding practices have helped to accomplish this. All of these practices require accurate knowledge of the nutrient requirement of the pig at each stage of development. With this knowledge, feeding multiple diets throughout growth and development, and at different stages of reproduction minimizes over formulation that occurs when one diet is fed over multiple phases. Simple feeding practices such as phase feeding and split sex feeding have greatly reduced nutrient excretion with little or no cost to the producer. In fact, in many cases these practices have improved profitability.

However, these feeding practices alone have not been enough to fully address regulatory concern over potential environmental pollution problems from large scale swine farms. Therefore, nutritionists have been searching for dietary additives which help to maximize dietary nutrient utilization and minimize excretion. One strategy for doing this has been to add enzymes to the diet to break down anti-nutritive factors in the diet. Nitrogen and P are the two nutrients

that are currently of greatest environmental concerns in swine production. Nitrogen from plant feed sources is generally 80 to 95% digestible (NRC, 1998), but it is fed to swine in high concentrations to meet amino acid requirements of the pig. Fresh swine manure contains approximately 6.1 g of N per kilogram of wet manure (Barker and Zublena, 1996). Phosphorus is required at much lower concentrations in the diet (.40 to .70%; NRC, 1998), but plant P is highly unavailable to the pig because the majority exists as phytate P (Nelson et al., 1968; Eeckhout and De Paepe, 1994; Pointilliart, 1994; NRC, 1998). Phytate is the salt of phytic acid. Phytic acid is a myoinositol ring with a phosphate group attached at each carbon (Figure 1). As a result of this high phytate P content, the P bioavailability from corn is estimated to be 14% (Cromwell, 1992; NRC, 1998) and the P bioavailability from soybean meal is estimated to be 31% (Cromwell, 1992; NRC, 1998). It is important to realize that these estimates of bioavailability are relative values using monosodium phosphate as a reference standards and not absolute bioavailabilities. As a result of these low bioavailabilties, P concentrations in swine manure average 2.034 g per kilogram of wet manure (Barker and Zublena, 1996).

Nelson et al. (1968) demonstrated that the addition of phytase to broiler diets increased the retention of phytate P. Phytases, are acid phosphatases that catalyze the hydrolysis of phosphate from phytate. Unfortunately, while this early work of Nelson et al. (1968) showed promising results, it was not cost effective to add the enzyme to swine or poultry diets. Recently, improvements in fermentation systems and technological advancements in molecular biology have allowed for microbial phytase to be produced in a cost-effective manner. As a result, a renewed interest in the addition of microbial phytase to swine diets has occurred over the last decade. Because phytic acid has the potential to carry up to two negative charges per phosphate group at a neutral pH (Erdman et al., 1979), it may form insoluble complexes with di- and tri-

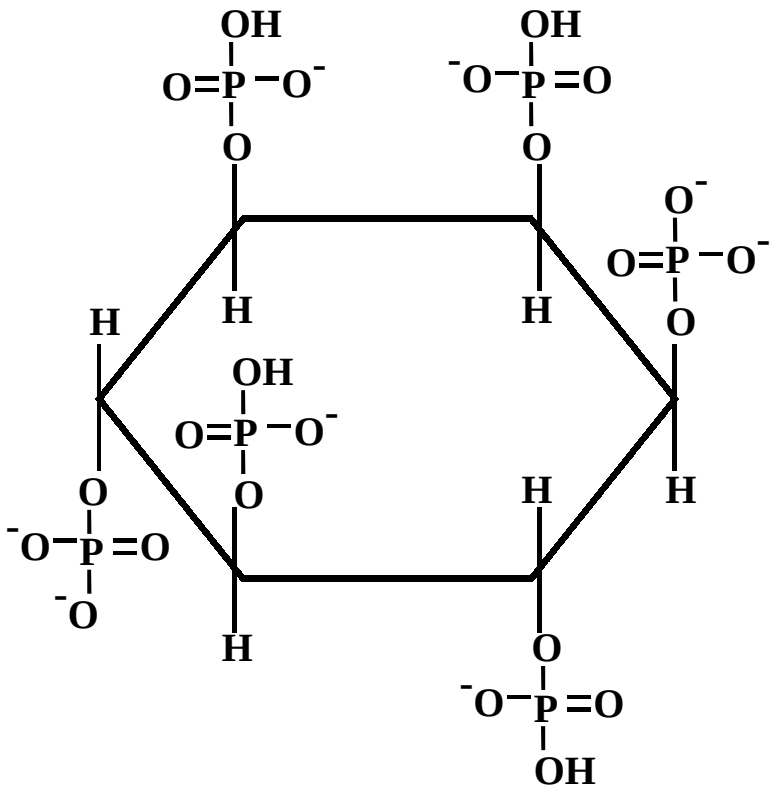

Figure 1. Phytic acid.

valent cations, sugars, and amino acids or peptides (Figure 2). Therefore, in addition to the effects of phytase on P digestibility, interest has grown on its effects on mineral, energy, and amino acid digestibilities.

Three studies from this dissertation focused on the effects of microbial phytase addition to swine diets on amino acid digestibilities. In the first study (Exp. 1, Chapter IV) the effects of microbial phytase in corn-soybean meal based diets were investigated and equivalency values of phytase for individual amino acids were generated. In the other two studies (Exp. 2, Chapter V and Exp. 3, Chapter VI) the effects of microbial phytase on amino acid digestibilities from alternative feedstuffs were investigated. A positive effect of phytase on amino acid digestibilities was observed in Exp. 1 and 3, but not in Exp. 2. The lack of a response in Exp. 2 could be due to the diet types fed or due to the lower phytase activities in these diets. The diet types fed in Exp. 2 may have affected phytase efficacy, but it seems unlikely that they were responsible for the total lack of a response since a corn-soybean meal based diet was one of the diet types, and a beneficial effect of phytase on amino acid digestibilities in corn-soybean meal based diets was observed in Exp. 1. In Exp. 2, the assayable phytase levels were approximately half of the calculated values. Therefore, the response to phytase would be expected to be lower and may have been below the detectable limit of the experiment.

In Exp. 1, pigs fitted with steered ileo-cecal valve (SICV) cannulas were fed either 10, 11, or 12% CP diets with no added phytase, or a 10 % CP diet with 250 or 500 U of added phytase per kilogram of diet. Feeding multiple levels of dietary CP and multiple levels of phytase at a low level of dietary CP allowed for calculation of CP/amino acid and phytase response curves. Increasing the level of dietary CP or adding phytase to the low P diet resulted

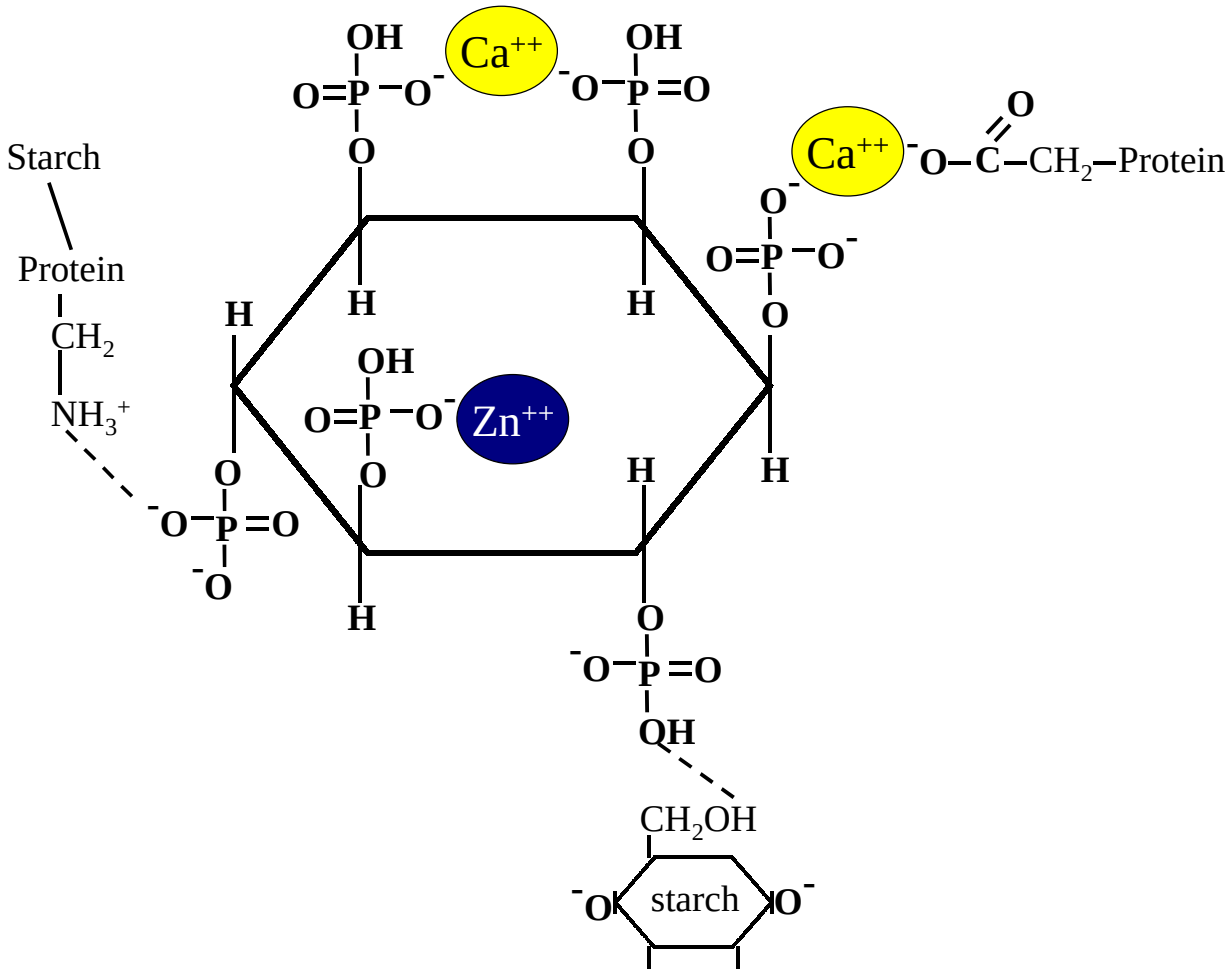

Figure 2. Mineral, amino acid, and carbohydrate chelating ability of phytic acid.

in an increased apparent ileal digestibility (AID) of most of the amino acids measured. Typically this type of experimental design has been used to determine the P equivalency value of microbial phytase (Yi et al., 1996; Kornegay et al., 1998; Radcliffe and Kornegay, 1998). Pigs are fed either increasing levels of P or phytase to a low P diet and response curves for phytase and P are generated. When this is done, the addition of P to the low P diet results in an increased AID and an increased apparent total tract (ATTD) of P. Phosphorus is added to the low P diet by increasing the amount of inorganic P in the diet. The P bioavailability of P from inorganic phosphates has been estimated to be 76.7% (Kornegay et al., 1998), while the P bioavailability of a corn-soybean meal based diet is typically 15 to 25% (calculated from NRC, 1998). Therefore, it is logical for the ATTD of P to increase when inorganic P is added to a low P diet. However, in Exp. 1 where similar treatments were used to create a CP response curve, the increase in CP digestibility seen with increasing dietary CP level was not due to the addition of an ingredient with a higher CP bioavailability. Instead, CP levels in the diet were reduced from the highest level of CP fed by adding a mixture (50:50, wt:wt) of corn starch and dextrose. Therefore, in all diets, corn and soybean meal were the only ingredients contributing to the CP pool. As a result, the decrease in CP and amino acid digestibilities seen as the level of dietary CP was reduced were due to either a negative effect of the corn starch-dextrose mixture on amino acid digestibility or to the relative contributions of endogenous N collected from ileal digesta. Reducing dietary CP levels by adding corn starch and dextrose to the diet, also resulted in an increased energy content of the diets.

Calculated metabolizable energy content of diets formulated to contain 12.0, 11.1, and 10.2% CP were 3453, 3483, and 3506 kcal/kg. The lysine to energy ratio has been investigated in pigs. Pigs eat to meet their energy requirement (NRC, 1998). As the level of energy in the

diet increases, feed intake decreases. Therefore, the nutrient density of the diet needs to increase as the dietary energy content increase to meet the nutritional requirements of the pig. However, pigs in Exp. 1 were limit fed, hence, feed intake should not have been a contributing factor.

Pigs secrete an average of 22.1 to 29.4 g of endogenous N per day (Auclair, 1986, reviewed by Schulze, 1994). Secretions of N which contribute to these endogenous losses include: salivary and gastric secretions (2.0 to 3.3 g/d), pancreatic secretions (2.5 to 6.7 g/d), biliary secretions (1.8 to 3.0 g/d), secretions from the small intestine (14.4 g/d), and sloughed intestinal cells (1.4 to 2.0 g/d) (Auclair, 1986, reviewed in Schulze, 1994). Between 73 and 79% of endogenous N secretions are reabsorbed by the small intestine (Krawielitzki et al., 1990; Soufrant et al., 1993). If it is assumed that pigs secrete an average of 25.75 g of N per day and that 75% of this N is reabsorbed, then approximately 6.44 g of endogenously secreted N reach the terminal ileum each day. This equates to approximately 40.25 g of CP (6.44 g of N x 6.25) per day. Nitrogen intake for pigs fed 12.0, 11.1, and 10.2% CP diets averaged 39.9, 37.2, and 35.7 g/d, respectively. This equates to 249.4, 232.5, and 223.1 g of CP per day for pigs fed 12.0, 11.1, and 10.2% CP diets. If the true ileal CP digestibility were 88% for all diets, then the amount of CP reaching the terminal small intestine would be 36.37, 34.34, and 33.21 g, respectively for pigs fed 12.0, 11.1, and 10.2% CP diets. If the apparent ileal digestibilities are then calculated by adding endogenous CP loss reaching the terminal ileum (40.25 g/d) then the apparent ileal digestibility of CP would be 71.86, 70.69, 69.96%, respectively for pigs fed 12.0, 11.1, and 10.2% CP diets. This represents a 2 percentage unit decrease in AID as a result of endogenous N losses when the CP content is decreases from 12.0 to 10.2%. The observed decrease was larger (7.1 percentage units based on N digestibility, and 3.5 percentage units based on calculating CP digestibility) using the sum of assayed amino acid concentrations in feed and

ileal digesta. Therefore, it appears that part of the decrease in CP and amino acid apparent ileal digestibilities observed when the level of dietary CP was reduced were due to something other than changes in the relative concentration of amino acids at the terminal ileum, or that a larger endogenous loss occurred than as estimated by Auclair (1986, reviewed in Schulze, 1994). Therefore, the protein response curve calculated in Chapter IV is biased by endogenous amino acid losses. However, since endogenous amino acid losses are not known for this study, it is impossible to adjust values to true ileal digestibilities.

As discussed in Chapter IV (Exp. 1), a 12% CP, corn-soybean meal based diet contains enough phytate to decrease CP digestibility by .013 percentage units if each phytate molecule binds one amino acid. Based on CP and phytase response curves for percent CP digestibility, 500 U of phytase per kilogram of diet is equivalent to .53 percentage units of CP. However, deriving CP response curves based on the percent of CP digested may provide misleading results, since different levels of CP were fed. Therefore, it is more useful to develop response equations for varying CP levels based on the amount of CP digested per kilogram of diet (g/kg of diet). Based on this equation from Exp. 1, 500 U of phytase can replace .150 percentage units of CP in the diet. Previous work has demonstrated that the addition of phytase to pig diets improves CP and/or amino acid digestibilities (Officer and Batterhan, 1992; Khan and Cole, 1993; Mroz et al., 1994; Kemme et al., 1997; Zhang and Kornegay, 1999). Estimated equivalency values of phytase for CP based on these studies has ranged from .29 percentage units for 900 U of phytase per kilogram of diet to 1.8 percentage units for 1,000 U of phytase per kilogram of diet. With the exception of Zhang and Kornegay (1999), all of the above studies fed only one level of phytase and one level of dietary CP. Therefore, extrapolations of the equivalency value of phytase at different levels could not be made and the bioavailability of CP

from the different diets was not adjusted for. Zhang and Kornegay (1999) investigated the effects of adding phytase to the diet on the AID of amino acids in growing-finishing pigs. During the finishing phase, diets were identical to those used in Exp. 1. Ileal digesta was collected at the end of the trial using the slaughter technique. Based on their findings, they estimated that 500 U of phytase per kilogram of diet could replace 1.01 percentage units of CP in the diet. Response equations for CP digestibility when the level of CP in the diet was increased in their study were based on CP digestibility as a percent and therefore, were biased as discussed above.

In Exp. 2 (Chapter V), SICV cannulated pigs were fed corn-soybean meal, corn-soybean meal-wheat middlings, or corn-soybean meal-meat and bone meal based diets with or without supplemental phytase (500 U/kg of diet). The addition of phytase to all diet types had no effect on the AID of CP or amino acids. The ATTD and AID of Ca and P were improved when phytase was added to the diet. Assayed phytase activity in all diets was lower than calculated. This may have contributed to the lack of an affect of phytase on amino acid digestibilities. Other dietary factors which may have influenced the efficacy of added microbial phytase were the high level of endogenous plant phytase in wheat, and the high bioavailability of P from meat and bone meal.

In Exp. 3 (Chapter VI), 12 crossbred pigs fitted with SICV cannulas were fed corn-wheat-soybean meal, corn-wheat-canola, or sorghum-corn-soybean meal based diets with or without the addition of 500 U of phytase per kilogram of diet. The addition of phytase to all diet types led to numerical improvements in the AID of all amino acids measured with many of these differences being significantly different. Improvements in the AID of amino acids ranged from .88 to 1.96 percentage units with the average being 1.47 percentage units. Total amino acid AID

was improved by 1.13 percentage units when 500 U of microbial phytase were added per kilogram of diet. This value is lower than reported in Exp. 1, where the addition of 500 U of phytase per kilogram of diet resulted in an improvement in the total AID of amino acids of 2.6 percentage units. The lower value observed in Exp. 3 is the result of several factors. First, in Exp. 1, the basal diet to which phytase was added was very low in CP (10.2%), compared to the diets fed in Exp. 3 (13.5%) which were much closer to meeting the CP requirement of the pig. Second, in Exp. 1, the only diet type fed was corn-soybean meal based. In Exp. 3, diet types included corn-wheat-soybean meal, corn-wheat-canola, and sorghum-corn-soybean meal based diets. Wheat is known to contain high levels of endogenous phytase. As a result, the efficacy of supplemental phytase would be expected to be lower.

In Exp. 4, the addition of β-mannanase to swine diets was investigated. Phytase, is undoubtedly one of the most effective enzymes discovered so far for combating antinutritive compounds in the diet. However, phytate is only one of many anti-nutritive compounds in diet typically fed to pigs. β-mannans represent another category of anti-nutritive compounds in swine diets. The initial interest in these compounds came from two very different areas. First, there was interest in feeding guar gum to poultry (Nagpal et al., 1971; Verma and McNab, 1982; Patel and McGinnis, 1985). When this was done an overall decreased performance was observed which was attributed to the high β-mannan content of guar gum. Second, β-mannans were investigated as a possible treatment for persons with non-insulin dependent diabetes (Rainbird et al., 1984; Sambrook and Rainbird, 1985; Todd et al., 1990). Ironically, the anti-nutritive properties of β-mannans that resulted in decreased performance of poultry fed diets containing guar gum actually are beneficial to persons suffering from non-insulin dependent diabetes.

Non-ruminants do not produce the enzyme β-mannanase which is needed to break the β-linked mannose backbone of β-mannans. Therefore, these sugars are unavailable for absorption. In addition, β-mannans have a high water holding capacity which results in an increase cell sloughing in the small intestine as a result of water movement out of the enterocytes. The combination of these factors has been shown to lead to decreased weight gains, decreased digestibilities of dietary amino acids, and an increased endogenous amino acid loss (Couch et al., 1967; Verma and McNab, 1982; Low and Rainbird, 1984; Rainbird et al., 1984; Patel and McGinnis, 1985; Sambrook and Rainbird, 1985; Brown et al., 1988; Hahn et al., 1995; Anonymous, 1999). The addition, of β-mannanase to swine and poultry diets has been shown to counteract some or all of these anti-nutritive effects of β-mannans. Corn contains only trace amounts of β-mannans, while soybean meal contains 1.22 to 1.48% β-mannan on a DM basis. In Exp. IV (Chapter VII) the addition of .05% Hemicell (β-mannanase) to grow-finish pigs fitted with SICV cannulas led to an increased ATTD of energy, and a numerical improvement in the AID of all amino acids measured. This is in agreement with the study from the Taiwan Sugar Company (Anonymous, 1999) where an improvement in ATTD energy digestibility was reported when Hemicell was added to growing-finishing diets. More recently, Pettey et al. (1999, 2000) reported an increased ADG in both nursery and growing-finishing pigs when Hemicell was included in the diet. No studies to date, except for the one reported in this dissertation, have investigated the effects of Hemicell on amino acid digestibilities. While the improvements were not significant, it is important to note that the AID of all amino acids measured was numerically improved at both CP levels fed when Hemicell was added to the diet. Many of these numerical differences approached significance. A small change in CP digestibility could lead to a substantial savings in diet cost.

In summary, the swine industry is rapidly changing. To survive in the industry, individuals and companies must be flexible and able to change as economic trends and regulatory policy dictates. One of the major issues facing the swine industry today is how to minimize the environmental impact of large scale swine production systems. Public concern over the environmental impact of swine production ranges from concerns over odor from these farms, to leakage from lagoons, to over application of nutrients to the land receiving manure application, to acid rain. To minimize these potential problems there are two basic strategies which can be followed. First, better manure handling and application procedures need to be adopted. Second, nutritionally it may be possible to increase utilization of dietary components and thereby decrease nutrient excretion. Ultimately, it will require a combination of these two strategies to effectively solve the problem.

This dissertation focused on two enzymes which when added to the diet help to breakdown anti-nutritive compounds in the diet, and therefore, increase nutrient digestibility. In both cases, it is obvious that these enzymes not only increase the digestibility of nutrients that are part of the target anti-nutritive compound, but they also indirectly increase the digestibility of other nutrients in the diet.

For producers to use these enzyme additives in their diets, it must be cost effective for them to add these products. Therefore, the economic benefit of adding these products must outweigh the cost of adding the product to the diet. The microbial phytase product used in the these experiments was Natuphos. Currently, BASF sells the Natuphos5000 product, which contains 5000 U of phytase activity per gram, for $18.15 per kilogram. The recommended inclusion rate for Natuphos in pig diets is 500 U per kilogram of feed. Therefore, 100 grams (1,000 kg x 500 U/kg ÷5000 U/g) of Natuphos5000 must be added per metric ton of feed to

supply 500 U of phytase activity per kilogram. Adding this amount costs $1.86/metric ton (100g ÷ 1000g x $18.15). The primary benefit from adding phytase is an increased P digestibility, and therefore a decreased need to add inorganic P. Company recommendations from BASF indicate replacing .11% of the total dietary P when 500 U of phytase are added per kilogram of diet. Some published and some unpublished research from our laboratory (Yi et al., 1996c; Harper et al., 1997; Radcliffe and Kornegay, 1998; Skaggs, 1999) and other laboratories (Jongbloed et al., 1996; review by Kornegay et al., 1998) indicates that the value is somewhat lower than that. If phytase can replace .10% of the P in a ration, then the cost savings per ton of feed is approximately $1.57/ton if dicalcium phosphate (DCP) is the inorganic P source used. This was calculated as follows:

Amount of P removed:	.10%
P content of DCP:	÷ 18.5%
Amount of DCP which can be removed:	.0054
Cost of DCP per metric ton:	x $291.00
Cost savings per metric ton:	$1.57

Therefore, based on these numbers alone, the addition of 500 U of phytase per kilogram of diet is costing $0.29 per metric ton ($1.86 - $1.57), and is therefore not cost effective. Obviously, if the price of DCP were to go up or the price of phytase were to go down, then it would become more cost effective. However, there are several other factors that need to be considered when calculating the economic benefits of adding microbial phytase. Natuphos5000 added at a level of 100 g per metric ton can replace .54% DCP or 5.4 kg of DCP creating 4.8 kg of additional "free space". This free space has a value, but it is difficult to calculate and will vary depending on individual situations. This free space will be filled in with corn and soybean meal. The result of this is that the energy content of the diet will increase, and therefore, less fat will need to be added to the diet to maintain the target dietary energy level.

In addition, adding phytase has been shown to affect more than just P digestibility. These non-phosphorus effects of phytase need to be considered when estimating the economical value of adding phytase to swine diets. In Exp. 1, it was estimated that the addition of 500 U of phytase per kilogram of diet could replace .15 percentage units of CP. The cost savings of replacing this CP, is dependent upon the cost of phytase and the plant ingredients used in the diet. Corn and soybean meal are the plant ingredients which are typically used in the United States for swine feed. In a 16% CP diet, if it is assumed that 500 U of phytase can replace .15 percentage units of CP, then the cost of the diet can be reduced by approximately $0.38 per metric ton by adding phytase and reducing CP levels. This estimate was calculated as follows:

Assumptions:

CP content of corn = 8.3%
CP content of SBM = 47.5%

Calculating the amount of corn and SBM needed to provide 16.0% CP without the addition of phytase:

Eq. 1: $.83X + .475Y = 16.0\%$
Eq. 2: $X + Y = 100$

Solving Eq. 2 for X yields:

$X = 100 - Y$

Substituting 100 - Y for X in Eq. 1 and solving for Y yields:

$.083(100 - Y) + .475(Y) = 16.0$
$.83 - .083Y + .475Y = 16.0$
$.392Y = 7.7$
$Y = 19.64$

Substituting 19.64 for Y in Eq. 2 and solving yields:

$X + 19.64 = 100$
$X = 80.36$

Therefore, the diet needs to contain:

80.36% Corn
19.64% SBM

Calculating the cost of the diet:

Prices:

Corn = $86.61/metric ton (Feedstuffs, 2000)
SBM = $187.00/metric ton (Feedstuffs, 2000)

Based on the above prices, the diet cost can be calculated as follows:

.8036 x $86.61 = $69.60
.1964 x $187.00 = $36.73
$106.33 per metric ton

By adding 500 U of phytase per kilogram of diet, the CP content of the diet can be reduced by .15 percentage units to 15.85%. The amount of corn and SBM needed to provide 15.85% CP is calculated as follows:

Eq. 3: $.083X + .475Y = 15.85\%$
Eq. 4: $X + Y = 100$

Solving Eq. 4 for X yields:

$X = 100 - Y$

Substituting 100 - Y for X in Eq. 3 and solving for Y yields:

$.083(100 - Y) + .475Y = 15.85$
$8.3 - .083Y + .475Y = 15.85$
$.392Y = 7.55$
$Y = 19.26$

Substituting 19.64 for Y in Eq. 4 and solving yields:

$X + 19.26 = 100$
$X = 80.74$

Therefore, the diet needs to contain:
80.74% Corn

19.26% SBM

Calculating the cost of the diet:

Prices:

Corn = $86.61/metric ton (Feedstuffs, 2000)
SBM = $187.00/metric ton (Feedstuffs, 2000)

Based on the above prices, the diet cost can be calculated as follows:

.8074 x $86.61 = $69.93
.1926 x $187.00 = $36.02
$105.95 per ton

The cost savings when phytase is added to the diet can be calculated as follows:

$106.33 - $105.95 = $0.38 per metric ton of feed

The cost savings of adding phytase to the diet and reducing the CP content is dependent on the amount of CP which can be replaced by phytase, and by the cost of corn and soybean meal. Table 1 shows the cost savings of adding phytase to the diet over a range of corn and soybean meal prices and at phytase equivalency values of CP ranging from .05 to .25 percentage units. In all cases, the addition of phytase did result in a reduction in the cost of the diet. This cost savings obtained from reducing SBM and increasing corn concentration in the diet needs to be weighed against the cost of adding phytase per ton of feed. A $0.38 decrease in grain costs per metric ton of feed does not justify spending $1.86 per metric ton to add phytase, but this savings combined with the $1.57 savings seen by replacing P, do make it cost effective to add phytase to the diet.

Hemicell, a β-mannanase, is a newer product and the pricing structure for it is still being developed. Based on the results seen in Chapter VII, Hemicell increases total tract energy

Table 1. Reduction in dietary corn and soybean meal costs when phytase is added to the diet to replace .05, .10, .15, .20, or .25 percentage units of CP.

Price per metric ton		Percentage unit reduction in CP when 500 U of phytase are added per kilogram of diet				
Corn	SBM	0.05	0.10	0.15	0.20	0.25
		----------Savings when phytase is added and CP is reduced----------				
$70.87	$165.35	$0.12	$0.24	$0.36	$0.49	$0.61
$70.87	$187.39	$0.14	$0.30	$0.44	$0.60	$0.74
$70.87	$209.44	$0.18	$0.35	$0.53	$0.71	$0.88
$70.87	$231.48	$0.21	$0.41	$0.62	$0.82	$1.03
$78.74	$165.35	$0.11	$0.22	$0.33	$0.44	$0.55
$78.74	$187.39	$0.14	$0.28	$0.42	$0.55	$0.69
$78.74	$209.44	$0.17	$0.33	$0.50	$0.66	$0.84
$78.74	$231.48	$0.20	$0.39	$0.58	$0.78	$0.97
$86.61	$165.35	$0.10	$0.20	$0.30	$0.40	$0.51
$86.61	$187.39	$0.13	$0.25	$0.39	$0.52	$0.64
$86.61	$209.44	$0.15	$0.31	$0.47	$0.63	$0.78
$86.61	$231.48	$0.19	$0.37	$0.55	$0.74	$0.93
$94.48	$165.35	$0.09	$0.18	$0.28	$0.36	$0.45
$94.48	$187.39	$0.12	$0.24	$0.35	$0.47	$0.60
$94.48	$209.44	$0.14	$0.30	$0.44	$0.58	$0.74
$94.48	$231.48	$0.18	$0.35	$0.53	$0.69	$0.87
$102.36	$165.35	$0.08	$0.17	$0.24	$0.32	$0.40
$102.36	$187.39	$0.11	$0.22	$0.33	$0.43	$0.54
$102.36	$209.44	$0.13	$0.28	$0.41	$0.55	$0.68
$102.36	$231.48	$0.17	$0.33	$0.50	$0.66	$0.83
$110.23	$165.35	$0.07	$0.14	$0.21	$0.29	$0.35
$110.23	$187.39	$0.10	$0.20	$0.30	$0.40	$0.50
$110.23	$209.44	$0.12	$0.25	$0.37	$0.51	$0.63
$110.23	$231.48	$0.15	$0.31	$0.46	$0.62	$0.77

digestibility, which could represent a significant savings to the producer. In addition, numerical increases were observed for the AID of all amino acids. If this effect is real, it would also lead to a significant cost savings. The addition of phytase (Simons et al., 1990; Beers and Jongbloed, 1992; Jongbloed et al., 1992; Kornegay and Qian, 1996; Yi et al., 1996c) or Hemicell (Pettey et al., 1999, 2000) in other studies have been shown to increase ADG. This is an additional benefit that would need to be quantified. Increases in ADG can be the result of an improved feed efficiency or due to an increased feed intake. An increase in feed efficiency has a direct effect on feed costs per animal. Increased feed intake with no change in feed efficiecny does not change the feed cost per animal, but it does result in animals reaching market weight sooner. This increased production capacity of facilities does have some economic benefit. All of these factors need to be considered when evaluating the economic benefits of a new feed additive.

To conclude, it is important to consider economic benefits of enzyme addition on a large-scale basis. A $0.38 savings per metric ton of feed when phytase is added and CP is reduced by .15 percentage units does not sound like a lot. However, assuming a feed:gain ratio of 2.8 for pigs up to 22.7 kg and a feed:gain ratio of 3.0 for pigs above 22.7 kg, the average pig consumes approximately 306.7 kg of feed to reach a market weight of 113.4 kg. As a result, the producer would save approximately $0.12 per hog going to market by adding phytase and reducing CP. Last year, approximately 83.77 million hogs were slaughtered in the United States. At a $0.12 savings per hog the industry could have saved nearly $10 million dollars on feed costs last year by adding phytase and reducing CP.

The primary driving force behind the interest in feeding enzymes such as phytase, has been concerns over the environmental impact of swine production. In the preceding discussion, the economic costs were weighed against the economic benefits of adding phytase. In addition

to the direct economic benefits as a result of reduced inorganic P supplementation and decreased CP content of the diet, the addition of phytase has the potential to bring economic benefits through environmental protection effects. The word potential is used, because in most areas these benefits cannot currently be realized by the producer. However, in the future the producer may realize direct economic benefit from environmentally friendly practices, or may benefit indirectly through decreased land requirements for manure application. The Netherlands is one of the few places where these economic benefits can be realized. Producers are not allowed to raise animals on their property at densities beyond their ability to apply manure N and P to available land. If more waste is produced than can be applied to the land to supply N and P as directed by the government, then the producer must pay a fine and pay to have the manure shipped elsewhere. Therefore, the addition of phytase to the diet allows a farmer to raise more animals on a given amount of land because less land is needed per animal to dispose of the waste.

Last year, in the United States, approximately 83.77 million hogs were slaughtered. If a feed:gain ratio of 2.8 for pigs up to 22.7 kg and a feed:gain ratio of 3.0 for pigs above 22.7 kg is assumed, then the average pig consumes approximately 306.7 kg of feed to reach a market weight of 113.4 kg. Assuming an average P content of the diet of .5% (.35 percentage units of P from plant sources and .15 percentage units from inorganic P sources), and a digestibility of plant P of 20% and of inorganic P of 76%, the average pig will excrete 3.2 g of P per kilogram of diet consumed (5 g/kg total P - ((.20 x 3.5 g/kg plant P) + (.76 x 1.5 g/kg inorganic P))). Therefore, the average pig excretes 981.4 g of P from weaning to market (3.2 g of P excreted x 306.7 kg of feed). If the addition of phytase results in an increase in P digestibility which allows for a .1 percentage unit reduction in the P content of the diet, then it also results in a 31.6%

reduction in P excretion (((5 g/kg total P - 1.84 g of P digested) - (4 g/kg total P-1.84 g of P digested)) ÷ 5 g/kg total P-1.84 g of P digested). Therefore, pigs fed diets containing 500 U of phytase per kilogram of diet would excrete 671.3 g of P (981.4 - .316 x 981.4) from weaning to market as opposed to 981.4 g. Each pig raised to market weight would excrete 310 g less P (981.4 g - 671.3 g) if phytase were added to the diet. If phytase were added to all swine diets in the United States, P excretion could be reduced by 25,968,700 kg per year based on last year's swine inventory.

If the same calculations are made for N excretion, assuming an average CP content of the diet of 16%, and an average apparent total tract digestibility of CP of 85%, and a reduction of dietary CP content by .15 percentage units when 500 U of phytase are added, then changes in N excretion can be estimated. Using the above assumptions, 1.18 kg of N would be excreted per hog fed a diet without phytase from weaning the market. Supplementing the diet with 500 U of phytase per kilogram of diet and reducing dietary CP content by .15 percentage units would result in a reduction in N excretion from 1.18 kg to 6.10 kg. This represents a reduction in N excretion of .08 kg per pig. Therefore, if phytase were added to all diets in the United States N excretion could be reduced by 6,701,600 kg per year based on last year's swine inventory.

The economic benefit of these reductions in N and P excretion is hard to quantify. To some producers, who have ample land, no realized economic benefit may occur. The economic environmental benefit is even harder to quantify. Certainly, if N and P are applied to the land at a level to meet crop needs, and not in excess, then the threat to the environment is minimized. Ultimately, this has an economic value. However, the question then becomes: Is the consumer willing to pay for the environmental benefit to offset producer costs? This is a question which has yet to be answered.

Concerns over the environmental impact of swine production will continue to grow in the future. The industry has changed from being a conglomerate of small farms to a very well organized, vertically integrated business. Therefore, the public does not have the same ties and relationship that it once had with farmers. As a result, there is more regulatory scrutiny over agricultural practices. In addition, the number of animals being raised per farm has increased dramatically. This has resulted in an increased manure production per farm. The industry needs to determine how to manage this waste in an environmentally friendly manner. In addition, nutritionists need to investigate diet options that minimize nutrient excretion. The pig will never be 100% efficient at utilizing all of the dietary nutrients. However, nutritionally the diet can be altered to optimize dietary nutrient utilization while minimizing the feed cost per pound of lean gain. Phytase, has proven to be a very effective dietary additive which improves not only P digestibility, but also Ca (Radcliffe et al., 1995), Zn (Pallauf et al., 1992; Lei et al., 1993c, Nasi and Helander, 1994), Mg (Pallauf et al., 1992; Nasi and Helander, 1994), Fe (Pallauf et al., 1992; Nasi and Helander, 1994), Cu (Pallauf et al., 1992; Nasi and Helander, 1994), CP (Mroz et al., 1991; Officer and Batterhan, 1992; Khan and Cole, 1993; Christensen and Nielson, 1995; Kemme et al., 1995; Jongbloed et al., 1995; Kornegay and Qian, 1996; Yi et al., 1996c; Zhang and Kornegay, 1999), and possibly energy digestibility. Hemicell, has not been as thoroughly researched, but evidence from this study and several others (Anonymous, 1999; Pettey et al., 1999, 2000) are very promising.

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Chapter IX

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Vita

John Scott Radcliffe, son of George and Jacquelyn Radcliffe, Jr., was born on September 10, 1973 in Cambridge, Maryland. He graduated from Cambridge South Dorchester High School located in Cambridge, Maryland in June of 1991. He received his Bachelor of Science in Animal Science at Virginia Polytechnic Institute and State University in June of 1995 at which time he began work towards his Master's degree in Animal and Poultry Sciences under Dr. E. T. Kornegay.

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