Calcium, Phosphorus, and Amino Acid Digestibilities in Low Phytate Corn by Growing Pigs

By

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Animal Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Abstract

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Nine growing barrows were used to determine apparent ileal digestibility coefficients (AID) and apparent total tract digestibility coefficients (ATTD) of calcium (Ca), and phosphorus (P) in low phytate corn (LPC), normal corn (NC), and soybean meal (SBM). The AID and the standardized ileal digestibility coefficients (SID) of CP and AA were also determined for these feedstuffs. Nine diets were formulated and fed to the animals in a 9 x 9 Latin Square design. Diets 1 and 2 contained LPC and NC, respectively, as the sole source of CP, AA, Ca, and P. Diets 3 and 4 were identical to diets 1 and 2 with the exception that limestone (iCa) and monosodium phosphate (iP) were added to these diets. Diet 5 contained SBM as the sole source of CP, AA, Ca, and P. Diet 6 was based on SBM with iCa and iP added. Diet 7 contained LPC, SBM, iCa, and iP, likewise, diet 8 contained NC, SBM, iCa, and iP. Diet 9 was a protein-free diet, which allowed for the calculation of endogenous losses of CP and AA. Diets 3, 4, 6, 7, and 8 were formulated to contain similar Ca:digestible P ratios. The results of the experiment indicated that the AID and ATTD of Ca and P were higher ($P < 0.05$) in LPC compared to NC. No differences

($P > 0.10$) were found when comparing AID of Ca and P to ATTD of Ca and P within the same diet. The AID of arginine (Arg), aspartate (Asp), glycine (Gly), isoleucine (Ile), lysine (Lys), phenylalanine (Phe), threonine (Thr), and valine were higher ($P < 0.05$) in LPC than in NC. The AID of all AA in SBM were higher ($P < 0.05$) than in both corns with the exception of alanine (Ala), cysteine (Cys), leucine (Leu), and methionine (Met). The SID of Lys, Phe, and threonine (Thr) were higher ($P < 0.05$) in LPC than in NC. The SID of Arg, histidine (His), and Lys were higher ($P < 0.05$) in SBM compared to both corns. This research indicates that LPC has a higher digestibility of Ca and P than NC and, therefore, less inorganic Ca and P need to be supplemented when feeding diets based on LPC rather than NC. The AA digestibility in LPC is at least as high as in NC. When measuring Ca or P digestibility at either the distal ileum or using total tract digestibility no differences were found, therefore, either method is an accurate depiction of what is digested by the animal.

Key Words; Pigs, Low phytate corn, Digestibility

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LIST OF ABBREVIATIONS

AA	Amino Acid(s)
Aaf	Amino Acid in feed
Aad	Amino Acid in digesta
AID	Apparent Ileal Digestibility coefficient(s)
ATP	Adenosine TriPhosphate
ATTD	Apparent Total Tract Digestibility coefficient(s)
Arg	Arginine
Ca	Calcium
CP	Crude Protein
Crd	Chromium in digesta
Crf	Chromium in feed
Cys	Cysteine
DM	Dry Matter
dP	Digestible Phosphorus
Fe	Iron

EAL	Endogenous Amino acid Losses
Glu	Glutamate
Gly	Glycine
His	Histidine
iCa	Limestone
iP	Monosodium Phosphate
Leu	Leucine
LPB	Low Phytate Barley
LPC	Low Phytate Corn
Lys	Lysine
Met	Methionine
Mg	Magnesium
N	Nitrogen
NC	Normal Corn
NRC	National Research Counsel
P	Phosphorus

Phe	Phenylalanine
Pro	Proline
SDSU	South Dakota State University
SEM	Standard Error of the Mean
SID	Standardized Ileal Digestibility Coefficient(s)
SBM	Soybean Meal
Thr	Threonine
Tyr	Tyrosine
Zn	Zinc

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CHAPTER 1

Introduction

Excess phosphorus (P) in swine manure has become a problem for proper land application. Typically, manure is applied according to nitrogen (N) requirements of the plant. This causes P to be incorporated in excess, which can lead to possible surface water pollution issues. The ratio of N:P is as important as the actual amounts of each excreted. By decreasing the amount of P excreted, the N:P ratio comes closer to the plants requirements. Recently a genetically altered low phytate corn (LPC) was developed to increase the amount of P that can be digested by the monogastric animal (Rayboy and Gerbasi, 1996). Low phytate corn has a lower percentage of P bound in the phytate complex compared to normal corn (NC). Normal corn has approximately 86% of P bound as phytate P (NRC, 1998), while LPC has approximately 35% of P phytate bound (Spencer et al., 2000), which is important because swine cannot digest phytate-bound P. Although the amount of phytate bound P varies between LPC and NC, the total percent of P is similar between both corn types. After its development, research was conducted to determine if LPC could reduce the amount of P excreted from swine. Spencer et al. (2000) found that not only did LPC reduce P excretion, but it also increased calcium (Ca) digestibility. Previously it was demonstrated that the digestibility of Ca, P, and AA are improved if exogenous phytase is added to a corn-soybean meal diet (Radcliffe et al., 1999; Stahl et al., 1999; Zhang and Kornegay, 1999). The reason for this increase is that Ca and AA are also bound in the phytate complex (Graf, 1986).

Therefore, it is reasonable to hypothesize that the AA digestibility in LPC is also higher than in NC. However, to the best of our knowledge, this hypothesis has not yet been tested in swine.

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CHAPTER 2

Calcium, phosphorus, and amino acid digestibilities in low phytate corn by growing pigs: Literature Review

1. Introduction

Since the recent development of genetically altered low phytate corn (LPC) there has been much attention given to the calcium (Ca) and phosphorus (P) digestibilities of the grain by growing pigs. It has been determined that LPC has higher Ca and P digestibility coefficients in growing pigs when compared to normal corn (NC) (Spencer et al., 2000; Sands et al., 2001; Veum et al., 2001). However, little research has been conducted to determine if other nutrients, such as AA, also have increased digestibility in LPC. The AA composition of LPC tends to be similar to that of NC (Spencer et al., 2000). Because some of the AA are bound in the phytate complex in NC, more AA could possibly be free and more digestible to monogastric animals in LPC. The AA digestibility in LPC can be determined by calculating apparent ileal digestibility coefficients (AID), and standardized ileal digestibility coefficients (SID) (Stein et al., 2001).

2. Calcium and phosphorus

Ca and P are macrominerals found in various locations throughout the body. Approximately 99% of the Ca in a pig's body is located in hard tissue (i.e. bones and teeth). The remaining 1% can be found in various soft tissues (Maynard and Loosli, 1962). Approximately 80% of the P is found in hard tissue, and the remaining 20% in soft tissue (Maynard and Loosli, 1962). Blood serum, ATP, phospholipids, coenzyme forms of certain B vitamins, and part of RNA/DNA are examples of soft tissue forms of P. With P being in many different locations, it serves many different functions. Although the primary function of both Ca and P is skeletal development, P also is important in cell membrane structure. P also plays a role in energy metabolism as a component of ATP.

2.1 Absorption and excretion of calcium and phosphrus

The mechanism of absorption of Ca and P are similar. Their absorption is dependent on their solubility in the small intestine where absorption takes place. Therefore, when held in a solution medium, both tend to be better absorbed.

2.1.1. Factors affecting calcium and phosphorus absorption

The absorption of Ca and P depends on at least five factors: 1) concentration of Ca and P in the diet, 2) source, 3) Ca:P ratio, 4) intestinal ph, 5) concentration of other nutrients (i.e. vitamin D, iron (Fe), magnesium (Mg) (Hayes, 1976; Crenshaw, 2000). The dietary concentration of Ca and P will influence the amount being absorbed by the animal. The source is more important for P than for Ca. The source of P is important because a portion of the P in cereal grains and vegetable proteins is bound in a phytate complex. The phytate form of P is cannot be readily digested by nonruminant animals because they lack the enzyme phytase, which makes the phytate P digestible to the animal. The amount of digestible P varies in different cereal grains and vegetable proteins (Patience and Thacker, 1989). The Ca:P ratio can also affect Ca and P absorption. Ca and P have an antagonistic relationship (Crenshaw, 2000). This relationship illustrates the importance of incorporating the proper Ca:P ratio in diets. For example a narrower Ca:P ratio (i.e. less than 1:1) yields a more efficient utilization of P (NRC, 1998). Intestinal ph also affects absorption of Ca and P. Other nutrients that can affect P absorption include vitamin D and Fe (Georgievskii et al., 1982). Intake of large quantities of aluminum, Fe, and Mg ions can cause P to be bound in insoluble forms, rendering them indigestible (Maynard and Loosli, 1962). Vitamin D also plays a role in the absorption of Ca and P, by stimulating intestinal absorption of both minerals (Hayes, 1976).

2.1.2. Method and control of calcium and phosphorus absorption

Ca and P are absorbed by both active and passive absorption with the majority of this absorption occurring in the duodenum of the small intestine (McDowell, 1992; Crenshaw, 2000). After being absorbed, Ca and P play a vital role in bone mineralization. Parathyroid hormone and calcitonin regulate the deposition and mobilization of Ca and P (Hayes, 1976). Deposition is important for growth and maintenance of the bone. The bones are not only necessary for the structure in the animal, but they also act as storage containers for Ca and P. This allows Ca and P to be mobilized from the bone when these minerals are deficient in other parts of the body (Maynard and Loosli, 1962).

The Ca and P that are not absorbed from the small intestine are passed further down the gastrointestinal tract and excreted in the feces. Ca and P of endogenous origin are excreted along with the unabsorbed Ca and P. Endogenous material is that which has been absorbed, metabolized, and deposited back into the gastrointestinal tract. The majority of this is being absorbed prior to the distal ileum, but some endogenous Ca and P are excreted in the feces. The main sources of endogenous P are salivary juice, gastric juice, biliary juice, pancreatic secretion, and sloughed mucosal cells (Fan et al., 2001)

2.2. Requirements of calcium and phosphorus

The amount of Ca and P needed by swine varies according to age and weight. The ratio of Ca:P is as important as the actual percentage of Ca or P in the diet. This ratio usually ranges from 1:1 to 1.5:1 (Cunha, 1977). The ratio is on a total Ca to total P basis. However, the total Ca to digestible P is a higher ratio. Table 2.1 shows the Ca and P requirements of growing pigs.

3. Amino acids

Amino acids are the monomers of proteins. Proteins serve many functions in the body, they make up hormones, enzymes, blood plasma proteins, milk protein, skeletal muscle, and immune antibodies. Proteins are present in muscle, skin, hair, and hooves (Pond et al., 1995). There are 23 AA commonly found in proteins. Ten of the 23 are indispensable for growing pigs, (i.e. they cannot be synthesized by the growing pig). The remaining 13 are dispensable.

3.1. Absorption and excretion of amino acids

Amino acid absorption occurs mainly in the duodenum and jejunum of the small intestine (Guyton and Hall, 1996). Absorption is accomplished by active transport.

There are at least two active transport systems; one for neutral AA and one for basic AA. The carrier system for AA is very specific. Changes such as removing charges on amino groups, adding side chains, etc. can prevent transport of AA. Some AA compete with one another for uptake (Pond et al., 1995). After absorption, AA can be utilized for tissue synthesis, growth, development of fetus, milk production, and for the synthesis of enzymes, hormones, and other metabolites. Amino acids in excess of the requirement are deaminated or transaminated and the carbon skeleton is used in metabolism while the amino group is excreted in the urine.

Not all AA are digested and absorbed. Undigested AA pass through the gastrointestinal tract and are excreted in the feces. There are also endogenous proteins deposited back in the gastrointestinal tract during metabolism. The main sources of endogenous protein are gastric juices, biliary juices, intestinal mucin, enzymes, albumins, sloughed cells, and saliva (Souffrant et al., 1993). Some of these proteins are digested and reabsorbed in the small intestine, while others will pass through the small intestine and enter the large intestine along with the undigested feed proteins. Some AA in the large intestine may be deaminated by the microbes. The carbon skeleton can then be utilized for energy by the microbes. Some nitrogen (N) may be absorbed in the large intestine as ammonia. It is then metabolized in the liver and excreted as urea via the urine, but most of the N from deaminated AA is excreted in feces along with N incorporation into microbial protein.

3.2. Amino acid requirements

The requirements for AA vary depending on sex, age, and stage of production of the animal. The AA requirement of pigs is similar to Ca and P in that the ratio is important as well as the specified quantity. The AA requirements of growing pigs are listed in Table 2.2.

4. Environmental Concerns

Nitrogen and P have become an environmental concern in the past few decades. Excess N and P in the environment have been associated with the enhanced eutrophication of surface waters, the Dead Zone in the Gulf of Mexico, and outbreaks of pfiesteria on the East Coast. With a large percentage of this excess N and P coming from non-point pollution sources, agriculture has been considered a main polluter because of the application of animal manure and inorganic fertilizer to farmland. After application and rainfall, P in has the potential to runoff or erode with the soil into nearby surface waters. This happens because of P's unique physical characteristics. Unlike N, which has a tendency to leach into groundwater, P binds tightly to soil. The soil can only hold a certain amount of P. Therefore during a rainfall, P may runoff in the water itself or with the soil to which it is tightly bound.

4.1. Swine manure

The typical production and composition of manure varies depending on type of facility, diet, water quality, etc. However in a liquid pit manure system for the growing pig, the production is typically 2000 liters of manure per year per pig space. This includes wash water and water spilled from water nipples. The nutrients produced per year in this type of situation are: total N = 7.71 kg/year; P_2O_5 (phosphate) = 6.35 kg/year; K_2O = 5.90 kg/year (Sutton et al., 1996). This composition becomes of interest when determining manure application rates for various crops. The ratio of N to P is 1.21:1 in the manure. The N:P ratio requirement for different crops varies. For example the N:P requirements of corn is 2.5:1 (Sutton et al., 1996). Therefore in order to meet the N requirements of corn when using manure as the sole fertilizer source, P is applied at over two times its requirement. This can lead to overload in the soil, and can cause environmental concerns.

There are two solutions to this problem. One is to apply manure according to P requirements instead of N requirements. An obvious drawback of this solution is that commercial N will need to be purchased and applied to meet the N requirements of the plant. Furthermore two times as much land will be needed for the application of a certain amount of manure. The second solution involves altering the composition of the manure so that it matches the plants requirements more closely. To accomplish this, the amount of P in the manure can be decreased. This can be accomplished by making sure that a larger proportion of the P in the feed is digested and absorbed by the pig. To do so, exogenous phytase or low-phytate sources of grain may be included in the diet.

5. Phytase research

Phytase is an enzyme that helps digest the phytate complex in cereal grain, making more P available to the animal. If the phytate (phytic acid) is not digested, it passes through the animal without degradation, thus increasing the P content of the manure.

5.1. Types of phytase

There are two types of phytase: 1) microbial phytase and 2) cereal phytase. Microbial phytase is found in the gastrointestinal tract of ruminant animals. All nonruminants lack microbial phytase, however microbial phytase can be produced by bacteria via fermentation and added to diets for the nonruminant animal. Some grains contain cereal phytase. Examples of this are wheat, barley, and rye. Feeding these grains can increase the amount of P digested by nonruminants and, thus, decrease the amount of P in the manure.

5.2. Effects of microbial phytase on phosphorus utilization

Harper et al. (1997) reported that 500 U/kg[1] of phytase used in a swine diet equaled approximately 0.87 to 0.96 g of P from inorganic sources. By including this amount of phytase in the diet for growing pigs, they estimated that fecal P excretion was reduced by 21.5%. These two facts are important. A decrease in inorganic P added to the diet will reduce the cost of the diet. Also, the decreased P excreted will move the N:P ratio towards the requirement of the crops. Harper et al. (1997) also found that increasing levels of supplemental phytase in swine diets resulted in a linear increase in the digestibility of P. Kornegay and Qian (1996) and Harper et al. (1997) also determined

[1] U/Kg is a measure of specific activity; the quantity of enzyme that liberates 1 *u*mol inorganic phosphate per minute from 5.1 mm-sodium phytate at ph 5.5 and 37^{o} C per kilogram of the diet.

that metacarpel ash and breaking force increased if diets marginal in P were supplemented with exogenous phytase.

5.3. Effects of phytase on the digestibility of nutrients other than phosphorus

Phytase not only helps digest P in the phytate form, but has also been shown to increase the digestibility of other nutrients. Zhang and Kornegay (1999) demonstrated that ileal AA digestibility coefficients in pigs increased with supplemented phytase except for proline (Pro) and glycine. The researchers also found that fecal P and Ca digestibilities increased with added phytase. They also estimated that 500 U/kg of phytase was the equivalency of 0.76 percentage units of CP. Radcliffe et al. (1999) reported that phytase increased the ileal digestibility in pigs of Ca, P, CP, and all AA except leucine (Leu), serine, Pro, methionine (Met), and tyrosine (Tyr). They estimated that 500 U/kg of phytase can replace 0.52 percentage units of CP.

Adeola et al. (1995) reported that plasma P and Mg concentration increased when phytase was added to a diet. They also observed that plasma zinc (Zn) concentrations improved when phytase was added to a diet containing no supplemental Zn. In addition, the Ca, P, and copper balance was improved with phytase addition. Stahl et al. (1999) reported that phytase can degrade phytate and release Fe from corn-soybean meal diets.

5.4. Effects of phytase on the digestibility of nutrients when fed to poultry

These effects are not limited to swine only. Ravindran et al. (1999) reported that exogenous phytase improved CP and AA digestibilities of various feedstuffs in broilers. Yi et al. (1996a) reported that adding phytase to turkey poult diets improved the ileal digestibility of all AA in a 22.5% CP diet. It was also reported that the amount of Zn retained in broilers improved by adding phytase to a corn-soybean meal based diet (Yi et al., 1996b).

In conclusion, phytase can improve the digestibility of AA, Ca, Fe, N, P, and Zn. It also has some effect on the digestibility of other microminerals by monogastic animals. The reason that these nutrients are better digested upon the addition of phytase to the diet is that they are bound in the phytate complex.

6. Low Phytate Grain Research

In recent years a genetically modified LPC has been developed. Low phytate corn is genetically altered to be homozygous for the 1pa 1-1 allele with 0.28% total P and 0.10% phytate P (Spencer et al., 2000). Two mutants of LPC were developed that contain 33% and 66% less phytate P than normal corn (Rayboy and Gerbasi, 1996), but both LPC and NC have similar amounts of total P. The only difference in the LPC and NC is that less phytate is found in LPC. Spencer et al. (2000) reported that there is 0.25% total P in NC and 80% of that is bound in the phytate complex. They also reported that the LPC had a total P of 0.28% with only 35% of it being in phytate P. With a smaller percentage of P in the phytate form in LPC, it would be reasonable to hypothesize that more P will be digested by pigs fed LPC compared to NC therefore, less P would be excreted in the manure. Two types of genetically altered low phytate barley (LPB) have been developed containing 13% and 43% phytate P (Rasmussen and Hatzack, 1998).

6.1. Effects of low phytate corn on nutrient digestibility

Spencer et al. (2000) reported that feeding LPC to growing pigs with no added P, increased digestibility and retention of P and reduced total P excretion compared to NC with no added P. This research illustrates that it is possible to decrease the inorganic P added to the diet and alter the N:P ratio of the manure, making it more suitable to be used as a crop fertilizer.

Sands et al. (2001) also reported that LPC increased P digestibility and retention and decreased the amount of P excreted compared to NC when fed to pigs. They also observed that N and Ca retention improved with pigs receiving LPC and phytase. Veum et al. (2001) found that LPC compared to NC reduced P excretion and increased P digestibility in growing pigs.

Spencer et al. (2000) reported that LPC has higher Ca digestibility than NC when fed to growing pigs. This occurred because the amount of Ca is the same in both corns, but less Ca is bound to the phytate portion in the LPC.

The digestibility by roosters of alanine, arginine, glutamate, Leu, lysine, Met, phenylalanine, and Tyr were higher in LPC compared to NC (Douglas et al., 2000). This indicates that less AA may be bound in the phytate complex of LPC than NC, therefore some AA maybe more digestible in LPC than in NC.

6.2. Low phyate barley

Poulsen et al. (2001) measured the nutritional value of low phytate barley (LPB) using rats as a model for swine and reported an improvement in apparent P digestibility in LPB than in normal barley. Low phytate barley fed to pigs can increase Ca and P digestibility and decrease P excretion in pigs compared to barley with higher phytate contents (Veum et al., 2002).

7. Methods for determining amino acid digestibility coefficients

There are several ways to measure digestibility coefficients of CP and AA. One approach is to calculate digestibility coefficients by dividing the amount of CP and AA excreted in the feces by the amount that was fed. Digestibility coefficients calculated in this manner are referred to as apparent total tract digestibility coefficients (ATTD). This approach assumes that all the CP and AA that was not in the excreta was absorbed by the animal. Some of the CP and AA, however, are utilized or altered by the microbes of the large intestine. Also, a portion of the CP and AA in the feces are of endogenous origin. For these reasons, ATTD are not a very accurate way of estimating CP and AA digestibility coefficients (Sauer and Ozimek, 1986; Sauer and de Lange, 1992).

7.1. Collection at the distal ileum

In order to receive a better estimate of the digestibility of CP and AA, researchers have developed procedures which allow for the collection of digesta at the distal ileum (Furuya et al., 1974; Decuypere et al., 1977; Stein et al., 1998). This allows the digesta to be collected before it comes in contact with microbes of the large intestine, thereby avoiding the microbial manipulation of digesta. This procedure involves inserting a T-cannula in the distal ileum of the animal allowing for partial or total digesta collections.

7.2. Using apparent and standardized digestibility coefficients to determine amino acid digestibility coefficients

There are several methods to determine AA digestibility. Two accepted methods are AID and SID (Tanksley and Knabe, 1984; Jondreville et al., 1995; Rademacher et al., 1999).

7.2.1. Apparent ileal digestibility

The AID are calculated by comparing what was fed to the animal with what was collected at the end of the ileum. An indigestible marker is added to the diet to calculate the amount of digesta collected in a partial collection. Two methods that may be used to measure apparent digestibility are: 1) the direct method; and 2) the difference method.

When using the direct method, an assay diet is formulated so that all the CP and AA in the diet come from a single feedstuff. The feedstuff is the only source of CP and AA in the diet, however it is not the only component of the diet. Other ingredients are included in the diet because the animal requires other nutrients. For example, to determine the AID of CP and AA in soybean meal (SBM) with the direct method, SBM would need to be the only feed ingredient containing CP and AA in the diet.

Feedstuffs that are low in CP and AA will have a high amount of endogenous protein relative to the amount in the diet (Fan and Sauer, 1994). This may cause the direct method to underestimate the digestibility of the feedstuff. Therefore, AID of CP and AA should be calculated in diets containing at least 16% CP. This presents a problem for many of the cereal grains, as they do not contain enough CP to formulate a diet in this manner. For such ingredients, the difference method may be used.

Measuring AID using the difference method requires formulating two diets. One diet has all its CP and AA from a sole feed ingredient high in CP and AA, such as SBM. A second diet contains a mixture of SBM and a test feed (i.e. corn). It is important that both diets contain similar CP levels. The AID is calculated for both diets by the direct method. The digestibility of the SBM from the first diet can then be subtracted from the second diet (corn-SBM) to determine the AID of corn by difference. The difference method may be a better measure of CP and AA digestibility than the direct method when using low CP feedstuffs, because the confounding effects of endogenous losses are offset by formulating a diet higher in CP, however this cannot be done with the low CP feedstuff itself (Fan and Sauer, 1995). The difference method tends to have a higher digestibility because the endogenous losses constitute a smaller portion of the nutrient collected (Fan and Sauer, 1995).

7.2.2. Standardized ileal digestibility

Neither the direct nor the difference approach take into account endogenous losses of CP and AA. By using these methods, the exact amount of the nutrient that is digested by the animal is not precisely determined. To do so it is necessary to determine the amount of endogenous AA in the digesta leaving the small intestine. This can be accomplished by capturing digesta from the distal ileum of animals fed a protein-free diet (Mitchell 1924). By subtracting the AA of endogenous origin from the total amount of AA captured at the distal ileum of pigs fed a protein containing diet, the SID of that diet is calculated (Rademacher et al., 1999; Stein et al., 2001). The SID should be the same regardless of the level of CP and AA from the test feed, because basal endogenous losses are taken into account.

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Table 2.1. Requirements of calcium and phosphorus in diets for growing pigs (NRC, 1998)

	Weight, kg					
Nutrient, %	3 - 5	5 - 10	10 - 20	20 - 50	50 - 80	80 - 120
Ca	0.90	0.80	0.70	0.60	0.50	0.45
P, total	0.70	0.65	0.60	0.50	0.45	0.40
P, available	0.55	0.40	0.32	0.23	0.19	0.15
Ca:P	1.29:1	1.23:1	1.17:1	1.2:1	1.11:1	1.13:1
Ca:digestible P	1.64:1	2:1	2.19:1	2.61:1	2.63:1	3:1

Table 2.2. Requirements of amino acids in diets for growing pigs[a] (NRC, 1998)

	Weight, kg					
Amino acid, %	3 - 5	5 – 10	10 - 20	20 - 50	50 - 80	80 - 120
Arginine	0.54	0.49	0.42	0.33	0.24	0.16
Histidine	0.43	0.38	0.32	0.26	0.21	0.16
Isoleucine	0.73	0.65	0.55	0.45	0.37	0.29
Leucine	1.35	1.20	1.02	0.83	0.67	0.51
Lysine	1.34	1.19	1.01	0.83	0.66	0.52
Methionine	0.36	0.32	0.27	0.22	0.18	0.14
Methionine + Cystine	0.76	0.68	0.58	0.47	0.39	0.31
Phenylalanine	0.80	0.71	0.61	0.49	0.40	0.31
Phenylalanine + Tyrosine	1.26	1.12	0.95	0.78	0.63	0.49
Threonine	0.84	0.74	0.63	0.52	0.43	0.34
Tryptophan	0.24	0.22	0.18	0.15	0.12	0.10
Valine	0.91	0.81	0.69	0.56	0.45	0.35

[a] Requirements are on a true ileal digestible basis calculated as {[intake – (excreted – endogenous)]/ intake} x 100%

Chapter 3

Calcium, phosphorus, and amino acid digestibility in low phytate corn by growing pigs

ABSTRACT: Nine growing barrows were used to determine apparent ileal digestibility coefficients (AID) and apparent total tract digestibility coefficients (ATTD) of calcium (Ca), and phosphorus (P) in low phytate corn (LPC), normal corn (NC), and soybean meal (SBM). The AID and the standardized ileal digestibility coefficients (SID) of CP and AA were also determined for these feedstuffs. Nine diets were formulated and fed to the animals in a 9 x 9 Latin Square design. Diets 1 and 2 contained LPC and NC, respectively, as the sole source of CP, AA, Ca, and P. Diets 3 and 4 were identical to diets 1 and 2 with the exception that limestone (iCa) and monosodium phosphate (iP) were added to these diets. Diet 5 contained SBM as the sole source of CP, AA, Ca, and P. Diet 6 was based on SBM with iCa and iP added. Diet 7 contained LPC, SBM, iCa, and iP, likewise, diet 8 contained NC, SBM, iCa, and iP. Diet 9 was a protein-free diet, which allowed for the calculation of endogenous losses of CP and AA. Diets 3, 4, 6, 7, and 8 were formulated to contain similar Ca:digestible P ratios. The results of the experiment indicated that the AID and ATTD of Ca and P were higher ($P < 0.05$) in LPC compared to NC. No differences ($P > 0.10$) were found when comparing AID of Ca and P to ATTD of Ca and P within the same diet. The AID of arginine (Arg), aspartate (Asp), glycine (Gly), isoleucine (Ile), lysine (Lys), phenylalanine (Phe), threonine (Thr), and valine were higher ($P < 0.05$) in LPC than in NC. The AID of all AA in SBM were higher ($P < 0.05$) than in both corns with the exception of alanine (Ala), cysteine (Cys), leucine (Leu), and methionine (Met). The SID of Lys, Phe, and threonine (Thr) were higher ($P < 0.05$) in LPC than in NC. The SID of Arg, histidine (His), and Lys were higher ($P < 0.05$) in SBM compared to both corns. This research indicates that LPC has a higher digestibility of Ca and P than NC and, therefore, less inorganic Ca and P need to be supplemented when feeding diets based on LPC rather than NC. The AA digestibility in LPC is at least as high as in NC. When measuring Ca or P digestibility at either the distal ileum or using total tract digestibility no differences were found, therefore, either method is an accurate depiction of what is digested by the animal.

Key Words; Pigs, Low phytate corn, Digestibility

Introduction

Phosphorus excretion in swine has become an environmental issue. A large percentage of P in conventional corn-soybean meal diets is bound in the phytate complex, which is a form that swine cannot digest. This leads to significant P excretion by the animal and possible environmental concerns. In recent years, novel low phytate grains have been developed (Rayboy and Gerbasi, 1996; Rasmussen and Hatzcack, 1998). These varieties have a higher digestibility of P compared to conventional grains. As an example, the P digestibility in low phytate barley (LPB) by rats is higher than in normal barley (Poulsen et al., 2001). Veum et al. (2002) reported that LPB fed to pigs can increase Ca and P digestibility and decrease P output compared to barley with higher phytate contents.

Low phytate corn is another grain that has been developed. Previous research has shown that LPC has greater Ca and P digestibilities compared to NC when fed to growing pigs (Spencer et al., 2000a; Sands et al., 2001; Veum et al., 2001). Because the LPC has a higher P digestibility than NC, less P is excreted in the feces. Douglas et al. (2000) reported that LPC had two to three times greater digestibility of P compared to NC when fed to chickens. It was also reported that the AA digestibility in LPC by roosters was similar or higher than in NC (Douglas et al., 2000). However to the best of our knowledge, no data on AA digestibilities of LPC by growing pigs are currently available.

The objective of the present experiment was to determine the digestibility coefficients of CP, AA, Ca, and P in LPC by growing pigs using different methodologies. A second objective was to compare the digestibility coefficients in LPC to those obtained in NC and soybean meal (SBM). A third objective was to compare AID of Ca and P to apparent total tract digestibility coefficients (ATTD) of Ca and P.

Materials and Methods

Animals, housing, and experimental design

Nine barrows (BW: 29.3 $\pm$ 1 kg) originating from the matings of duroc x pietrain x large white boars to york x duroc x landrace sows were equipped with a simple T-cannula in the distal ileum using procedures adapted from Stein et al. (1998). Following the surgery, pigs were housed individually in 1.2 x 1.4 m pens with partly solid, partly slatted floors. Room temperature was set at 22°C. Animal care procedures were approved by the South Dakota State University (SDSU) Animal Care and Use Committee (#00-A037).

A 9 X 9 Latin square design was used with 9 periods and 9 animals comprising the square. The animal was the experimental unit and each experimental period lasted nine days. After the commencement of the experiment, pigs remained in their respective pens until they reached normal slaughter weights at which time they were harvested at the Meat Science Laboratory at SDSU.

Diets and feeding

The LPC and the NC were ground to pass through a 0.3175 cm screen. The mill had a negative air-assist system minimizing the possibility of contamination. The nutrient composition of the two corns and SBM is shown in Table 3.1.

A total of nine diets were prepared (Table 3.2) and randomly assigned within the Latin square. Diets 1 and 2 contained LPC and NC, respectively. In diet 1, LPC was the sole source of CP, AA, Ca, and P. Similarly, the only feed ingredient contributing CP, AA, Ca, and P in diet 2 was NC. Diets 3 and 4 were similar to diets 1 and 2 with the exception that inorganic Ca and P were included in these diets. Therefore, the Ca and P concentrations in these diets correspond with current recommendations for growing pigs; i.e. 0.5% Ca and 0.2% digestible P (dP) (NRC, 1998). These levels were reached by including limestone (iCa) and monosodium phosphate (iP) in both diets. Diet 5 was a SBM-based diet in which SBM was the only feed ingredient containing CP, AA, Ca, and P. Diet 6 was a SBM-iCa-iP diet formulated to contain Ca and dP according to recommendations (NRC, 1998). Diets 7 and 8 were LPC-SBM-iCa-iP and NC-SBM-iCa-iP, respectively, formulated to contain Ca and dP as recommended (NRC, 1998). Diet 9 was a protein-free diet. Diets 3, 4, 6, 7, and 8 were formulated to contain similar Ca:dP ratios. In diets 5,6, and 9, Solka Flock – a synthetic source of fiber – was included to increase the total concentration of crude fiber. Dextrose and soybean oil were included in all diets at levels of 5% and 3%, respectively, to enhance palatability. Chromium oxide (0.25%) was included in all diets as an inert marker; vitamins, salt, and microminerals were included at levels that met or exceeded the NRC recommendations for growing pigs (NRC, 1998). The nutrient composition of the diets is shown in Table 3.3.

Table 3.4 gives an overview of the diets, their main use, and the method used to calculate nutrient digestibility. The apparent ileal digestibility coefficients (AID) and the ATTD of Ca and P were calculated for all diets with the exception of the protein free diet using the direct method. In addition, the AID and the ATTD of Ca in the LPC and the NC diets were determined using an adaptation of the difference method. The AID and the standardized ileal digestibility coefficients (SID) of CP and AA were determined for the LPC, NC, SBM, and the corn-SBM diets using the direct method. The AID of AA and CP in LPC and NC were also calculated using the difference method (Fan and Sauer, 1995).

Pigs were fed at a level of three times their maintenance energy requirement in two equal meals at 800 and 2000 h. The energy requirement of the animals on a daily basis was calculated using equation [1] (NRC, 1998):

$$106 \text{ kilocalories x kg of BW}^{0.75} \qquad \textbf{[1]}$$

Water was available at all times throughout the experiment.

Following the surgery, pigs were allowed a four-week recuperation period before the experiment was initiated. During this time, a standard corn-SBM based 16% CP grower diet was provided.

Data recording and sample collection

The initial 6 days of each period was considered an adaptation period to the diet. At the same time each morning on days 7 and 8, fecal samples were collected by grab sampling and immediately frozen. Ileal digesta were collected for 12 h on days 8 and 9 as described by Stein et al. (1999a). In short, a plastic bag was attached to the cannula barrel and digesta flowing into the bag were collected. Bags were removed whenever they were filled with digesta - or at least every 30 min - and immediately frozen at – 20°C to prevent any bacterial degradation of the digesta proteins. On the completion of each experimental period, the animals were deprived of feed overnight and the following morning, a new experimental diet was fed.

Chemical analysis

At the end of the experiment, fecal and ileal samples were thawed, mixed within animal and diet, and a sub-sample was taken for chemical analysis. A sample of each feedstuff and each diet was taken as well. Fecal and digesta samples were freeze-dried and all samples were finely ground prior to chemical analysis.

Protein analysis was performed on digesta samples, diets, and the protein containing feed ingredients (AOAC, 1990). Amino acids were analyzed on a Chrom-tech HPLC amino acid analyzer (Thermo Separation Products, San Jose, CA), using ninhydrine for post-column derivatization and nor-leucine as the internal standard (AOAC, 1995). Prior to analysis, samples were flushed with nitrogen and hydrolyzed with 6 *N* HCL for 24 hours at 110^{o}C. Methionine and cysteine were determined as methionine sulfone and cysteic acid after cold performic acid oxidation overnight prior to hydrolysis. The chromium (Cr) content of the diets, digesta, and feces was determined according to Fenton and Fenton (1979). After wet acid digestion with nitric and perchloric acids (2:1), Ca and P concentrations of the diets, digesta, and feces were determined. The Ca concentrations were determined by an atomic absorption spectrophotometer (model 5000 Perkin-Elmer, Norwalk, CT). The P determination was accomplished with a UV-vis scanning spectrophotometer (model UV-2101 PC Shimadzu Corporation, Kyoto, Japan).

Calculations and statistical analysis

The AID of AA in LPC, NC, SBM, and the corn-SBM diets were calculated using the direct method according to equation [2] (Stein et al., 1999a):

$$AID = (100 - [(AAd/AAf) \times (Crf/Crd)] \times 100 \qquad \mathbf{[2]}$$

where AID is the apparent ileal digestibility coefficient of an AA (%), AAd is the AA content in the ileal digesta DM, AAf is the AA content in the feed DM, Crf is the Cr content in the feed DM, and Crd is the Cr content in the ileal digesta DM. The AID of CP were calculated using the same equation. Likewise, this equation was also used to directly measure the ATTD and AID of Ca and P in the diets.

The basal endogenous flow to the distal ileum of AA was calculated based on the flow obtained after feeding the protein-free diet according to equation [3] (Stein et al., 1999b):

$$EAL = [AAd \times (Crf/Crd)] \qquad \textbf{[3]}$$

where EAL is the basal endogenous loss of an AA (mg per kg DMI), AAd is the AA content in the ileal digesta DM, Crf is the Cr content in the feed DM, and Crd is the Cr content in the ileal digesta DM. The basal endogenous loss of CP was determined using the same equation.

By correcting the AID for the basal endogenous losses of each AA, SID were calculated using equation [4] (Stein et al., 2001):

$$SID = [AID + EAL/AAf] \qquad \textbf{[4]}$$

where SID is the standardized ileal digestibility coefficient (%), AID is the apparent ileal digestibility coefficient (%) of an AA, EAL is the endogenous loss of an AA calculated using equation [3], and AAf is the concentration of that AA in the feed DM. The SID of CP were also calculated using equation [4].

The AID of CP and AA in LPC and NC were also determined using the difference method as described by Fan and Sauer (1995). The SBM and the corn-SBM diets were used for these calculations according to equation [5] (Fan and Sauer, 1995):

$$D_A = (D_D - D_B \times S_B)/S_A \qquad \textbf{[5]}$$

$$S_A = 1 - S_B$$

where D_A is the AID (%) of AA in the LPC or NC, D_D is the AID (%) in either the LPC-SBM or the NC-SBM diet measured using the direct method, D_B is the AID (%) of AA in

SBM measured using the direct method, S_B is the contribution level of an AA (decimal %) from the SBM in the LPC-SBM or the NC-SBM diet, and S_A is the contribution level of that AA (decimal %) from LPC or NC in the corn-SBM diets. The AID of CP in LPC and NC were calculated using the same equation.

The AID and the ATTD of Ca for the LPC and NC diets were calculated using an adaptation of the difference method. This was accomplished by determining the AID and the ATTD of iCa according to equation [5] using the SBM and the SBM-iCa-iP diets. The AID and ATTD of Ca in LPC and NC were then calculated according to equation [6]:

$$X\ (Y) + W\ (Z) = A, \text{ or} \qquad \mathbf{[6]}$$

$$W = [A-(X \times Y)]/Z$$

where W is the AID (%) of Ca in LPC or NC, A is the AID (%) of Ca in either the LPC-iCa-iP or the NC-iCa-iP diet, X is the AID (%) of iCa determined from diet 6 using equation [5], Y is the amount of iCa (decimal %) in the LPC-iCa-iP and NC-iCa-iP diets, and Z is the amount of LPC and NC (decimal %) in the LPC-iCa-iP or the NC-iCa-iP diet.

Data were analyzed statistically using the PROC MIXED procedure of SAS (Littell et al., 1996). An analysis of variance was conducted with diets as the fixed effect and pig and period as random effects. The Least Square Means were calculated and differences were determined with the PDIFF option of SAS. This procedure was also used for comparisons of Ca and P digestibility coefficients between the diets and for the comparison of CP and AA digestibility coefficients between the diets. However, when comparing AID of Ca and P to ATTD of Ca and P, a Student's t-test was used. A *P*-value of 0.05 or less was considered significant.

Results

All barrows remained healthy throughout the experiment and readily consumed the rations supplied to them.

Calcium and phosphorus digestibility

The apparent digestibility coefficients for Ca and P are shown in Table 3.5. Pig and period were found to have no effect ($P > 0.10$) on neither Ca nor P digestibility.

The AID and ATTD of Ca were higher ($P < 0.01$) in LPC than in NC. For neither LPC nor NC were the AID and ATTD affected ($P > 0.05$) by the addition of iCa. Likewise, the AID and ATTD for SBM were similar ($P > 0.05$) to the AID and ATTD in the SBM-iCa-iP diet. The AID of Ca in the two corn-SBM diets were similar ($P > 0.05$). The AID and ATTD of Ca in LPC were higher ($P < 0.01$) than in SBM; however, the AID and ATTD of Ca in NC and SBM were similar ($P > 0.05$).

There were no differences ($P > 0.15$) between AID and ATTD of Ca within any of the diets.

The AID and the ATTD of P in LPC were higher ($P < 0.01$) than in NC. All diets that were formulated to contain identical concentrations of dP had similar ($P > 0.05$) AID and ATTD of P. The AID and the ATTD of P in SBM were higher ($P < 0.05$) than in NC, but lower ($P < 0.05$) than in LPC. The AID and the ATTD of P in NC and SBM increased ($P < 0.05$) if iP was added, however this was not the case for LPC.

There were no differences ($P > 0.30$) between AID and ATTD of P within any of the diets.

Crude protein and amino acid digestibility

For all calculations, pig and period were found to have no effect ($P > 0.10$) on the AID and the SID of CP and AA. The AID and SID of CP and AA measured by the direct method for the LPC, NC, SBM, and the corn-SBM diets are shown in Tables 3.6 and 3.7, respectively.

The AID of arginine (Arg), aspartate (Asp), glycine (Gly), isoleucine (Ile), lysine (Lys), phenylalanine (Phe), threonine (Thr), and valine (Val) were higher ($P < 0.05$) in LPC compared to NC. Soybean meal had higher ($P < 0.01$) AID of all indispensable AA except leucine (Leu) and methionine (Met) compared to LPC and NC. However, the AID of AA in the corn-SBM diets were similar ($P > 0.05$). The AID of all dispensable AA, with the exception of alanine (Ala) and cysteine (Cys), were higher ($P < 0.01$) for SBM than in LPC and NC.

The SID of Lys, Phe, and Thr were higher ($P < 0.05$) for LPC than for NC, but for all other AA and for CP, there were no differences ($P < 0.05$) between the corns. The SID of Arg, histidine (His), and Lys were higher ($P < 0.05$) in SBM than in LPC and in NC. The SID of Ile and Thr in SBM were similar ($P > 0.10$) to LPC, but higher ($P < 0.05$) than in NC. The SID of Leu and Ala were higher ($P < 0.05$) in LPC than in SBM. However, for the remaining AA, no differences in SID ($P > 0.05$) between SBM and LPC and NC were found. The corn-SBM diets had similar ($P > 0.05$) SID of all AA regardless of the type of corn used in the diet.

The basal endogenous losses of CP and AA were calculated based on collections of digesta from pigs fed the protein-free diet (Table 3.8).

The AID of CP and AA in LPC and NC were calculated using the difference method (Table 3.9). When digestibility coefficients of CP and AA were calculated using the difference method, the LPC was found to be similar ($P > 0.15$) to NC.

Discussion

Calcium and phosphorus digestibility

In the present experiment, the digestibility coefficients by growing pigs of Ca in LPC and NC were compared. The digestibility coefficients were measured in diets in which LPC or NC was the sole source of Ca. To the best of our knowledge, such a comparison has not been previously reported. The LPC had higher Ca digestibility coefficients than NC because less Ca is bound in the phytate complex of LPC compared to NC. Therefore, more Ca from LPC can be digested and absorbed compared to NC. The fact that the digestibility coefficients of Ca in NC and SBM were similar indicates that similar proportions of Ca are bound in the phytate complex in each of the two feed ingredients. However, LPC has less Ca bound in the phytate complex compared to both SBM and NC. Because no increases in Ca digestibility occurred when iCa was added to LPC, NC, or SBM, it appears that Ca digestibility of iCa may be lower than that of LPC, but higher than SBM and NC.

The digestibility coefficients of Ca in the LPC-SBM-iCa-iP and the NC-SBM-iCa-iP diets determined in the present experiment are similar to the values reported by

Spencer et al. (2000b) and Veum et al. (2001). However, the values obtained in the current experiment were lower than the digestibility coefficients of Ca in corn-SBM based diets reported by Sands et al. (2001). In the research conducted by Sands et al. (2001), higher concentrations of inorganic Ca were included in the experimental diets compared to the current experiment, which may explain this difference.

The data obtained in this experiment indicate that there is no net absorption or net excretion of Ca in the large intestine. This observation is in agreement with previous published data (Partridge, 1978). Based on these findings, there seems to be no advantage of measuring Ca digestibility coefficients at the distal ileum rather than using total tract digestibility coefficients.

The digestibility coefficient of P in LPC is approximately two times that of P in NC. This result was expected because less P is bound in the phytate complex in LPC than in NC. Therefore, the pig can digest a larger percentage of the P in the corn.

The digestibility coefficients of P in the two corn-SBM diets used in this experiment are similar to the values reported by Spencer et al. (2000b) and Veum et al. (2001).

The digestibility coefficients of P determined directly in LPC of the current experiment are similar to the values obtained using an invitro procedure (Spencer et al., 2000b; Veum et al., 2001). However, the digestibility of P in NC calculated in the current experiment is higher than values for NC reported by Spencer et al. (2000b) and Veum et al. (2001) and also higher than the NRC-value (NRC, 1998). The reason for this difference is not known. However, it may be because a different methodology (i.e. AID of P measured in live animals) was used in the present experiment, while in vitro procedures were used in the other experiments. The P digestibility of SBM determined in the current experiment is similar to that reported by NRC (NRC, 1998).

Partridge (1978) reported that there is no net absorption or net excretion of P in the large intestine in pigs. In the current experiment, similar ileal and total tract digestibility coefficients were calculated, thus confirming the findings of Partridge (1978). The true ileal digestibility coefficients and the true fecal digestibility coefficients of P have been reported to be similar in pigs fed SBM-based diets (Fan et al., 2001). The true digestibility coefficients of P are not confounded by the endogenous P. Because the output of endogenous P is higher when measured at the distal ileum rather than over the total tract, it was concluded that some absorption of endogenous P occurred in the large intestine (Fan et al., 2001). When calculating digestibility coefficients, the output at the distal ileum or in the feces is inversely related to the digestibility coefficient of a nutrient. In the work by Fan et al. (2001), the AID and the ATTD of P were calculated but not statistically compared. However, because of the higher endogenous P output at the distal ileum compared to the fecal output, the AID of P would have been lower than the ATTD if they had been compared. If that is correct, it would be expected that the diets in the current experiment that contained a low amount of P relative to the animal's requirement (i.e. the NC diet, and the SBM diet) would have a higher ATTD than AID of P because relatively more endogenous P would be present in ileal digesta than in the feces from the animals fed these diets. However, regardless of the dietary P-content, no differences were found when comparing the AID of P to the ATTD of P. The reason for the difference between the results of the two experiments may be that the direct method was used in the current experiment to measure the digestibility of P, whereas the regression method was used in the experiment conducted by Fan et al. (2001).

In conclusion, Ca and P are more digestible in LPC than in NC. This finding agrees with previously reported data (Spencer et al., 2000b; Veum et al., 2001). No differences were found when measuring Ca or P digestibility at the distal ileum compared to using total tract digestibility, which indicates that no net secretion or absorption of these minerals occurred in the large intestine.

Crude protein and amino acid digestibility

Previous research has indicated that AA are bound in the phytate complex (Biehl and Baker, 1996). Because less phytate is present in LPC than in NC, an improved AID and SID of AA in LPC might be expected. Previously, LPC was found to have an increased SID of some AA compared to NC when fed to roosters (Douglas et al., 2000). In the present experiment, the SID of Lys, Phe, and Thr by pigs were higher in LPC than in NC. The higher digestibility coefficients of some AA in LPC than in NC indicated that fewer AA are bound in the phytate complex in LPC than in NC. However, in the current experiment it was also determined that when each corn was mixed with SBM into a diet, no differences in digestibility coefficients of AA were found between the LPC-SBM and the NC-SBM diets. This occurred because the differences in digestibility coefficients of AA between the LPC and NC are too small to significantly impact the digestibility coefficients of AA in a corn-SBM based diet.

The AID and the SID of most AA in SBM and NC are within the range of values previously reported for these two ingredients (i.e. NRC, 1998; Rademacher et al., 1999; Stein et al., 1999a). The current experiment also showed that the SID of some AA were similar between SBM and NC, which agrees with previous work (Jondreville et al., 1995; NRC, 1998).

The endogenous losses measured in the current experiment are similar to the values reported by Rademacher et al. (1999) and Stein et al. (1999b).

In conclusion, LPC has some AA, which are more digestible than they are in NC. However, when either LPC or NC was mixed with SBM, no differences were found in digestibility coefficients of AA between the LPC-SBM and the NC-SBM diets.

Implications

Results of the present experiment indicate that Ca, P and some AA are more digestible by growing pigs in LPC than in NC. This indicates that less Ca and P from inorganic sources need to be supplemented to diets formulated with LPC than with NC and less Ca and P will be excreted from animals if they are fed LPC compared to NC. Both ileal and total tract digestibility coefficients can be used to predict the absorption of Ca and P. Low phytate corn also improved the digestibility of some AA compared to NC.

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Table 3.1. Nutrient composition of normal corn, low phytate corn, and soybean meal (DM basis)

Nutrient	LPC[a]	NC[a]	SBM[a]
DM	88.28	87.15	92.66
Calcium	0.01	0.01	0.45
Phosphorus	0.26	0.28	0.65
Crude protein	10.48	10.86	51.49
Indispensable amino acids			
Arginine	0.45	0.45	3.48
Histidine	0.27	0.28	1.22
Isoleucine	0.30	0.31	2.07
Leucine	1.02	1.08	3.63
Lysine	0.27	0.29	3.14
Methionine	0.25	0.23	0.82
Phenylalanine	0.42	0.44	2.38
Threonine	0.28	0.27	1.68
Valine	0.42	0.44	2.24

continued

Table 3.1. continued

Dispensable amino acids			
Alanine	0.65	0.66	2.07
Aspartate	0.59	0.60	5.61
Cysteine	0.16	0.15	0.56
Glutamate	1.66	1.67	9.18
Glycine	0.35	0.35	2.08
Proline	0.81	0.80	2.57
Serine	0.47	0.47	2.90
Tyrosine	0.36	0.37	1.71

[a] LPC = low phytate corn; NC = normal corn; SBM = soybean meal

Table 3.2. Ingredient composition (as is) of experimental diets

Item	Diets								
Ingredient, %	1	2	3	4	5	6	7	8	9
Low phytate corn	90.95	0	89.79	0	0	0	65.30	0	0
Normal corn	0	90.95	0	89.52	0	0	0	65.09	0
Soybean meal	0	0	0	0	37.65	37.65	24.50	24.50	0
Limestone	0	0	1.08	1.08	0	1	1.07	1.07	1.35
Monosodium phosphate	0	0	0.16	0.70	0	0.48	0.16	0.56	0.80
Solka flock[a]	0	0	0	0	2	2	0	0	3
Dextrose	5	5	5	5	5	5	5	5	5
Soybean oil	3	3	3	3	3	3	3	3	3
Chromium oxide	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.40	0.40	0.32	0.05	0.40	0.16	0.32	0.13	0.40
Vitamin premix[b]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Trace mineral premix[c]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Cornstarch	0	0	0	0	51.30	50.06	0	0	85.80

continued

Table 3.2. continued

[a] Fiber Sales and Development Corp. Urbana, OH

[b] Vitamin premix provided per kilogram of diet: 40,131 IU of vitamin A acetate; 3,969 IU of vitamin D^3 as d-activated animal sterol; 353 IU of vitamin E as alphatocopherol acetate; 6 mg of vitamin K as menadione dimethylpyrimidinol bisulfate; 1.59 mg of biotin; 203 mg of niacin; 99 mg of pantothenic acid; 40 mg of riboflavin; and 0.20 mg of vitamin B_{12}

[c] Trace mineral premix provided per kilogram of diet: 46 mg of copper; 220 mg of iron; 0.55 mg of iodine; 46 mg of manganese; 0.55 mg of selenium; and 228 mg of zinc

Table 3.3. Nutritional composition of diets (dry matter basis)

	Dietary treatments[a]							
Treatment No.:	1	2	3	4	5	6	7	8
Diet description	LPC	NC	LPC-iCa-iP	NC-iCa-iP	SBM	SBM-iCa-iP	LPC-SBM-iCa-iP	NC-SBM-iCa-iP
Item								
DM	90.53	89.68	89.04	88.89	91.62	91.81	89.73	90.00
Calcium	0.01	0.01	0.46	0.44	0.23	0.60	0.56	0.54
Phosphorus	0.27	0.26	0.32	0.52	0.27	0.43	0.41	0.51
Crude protein	8.95	8.67	8.31	8.96	19.67	19.07	22.70	21.14

continued

Table 3.3. continued

Indispensable amino acids								
Arginine	0.44	0.40	-	-	1.42	-	1.21	1.20
Histidine	0.25	0.23	-	-	0.52	-	0.51	0.50
Isoleucine	0.29	0.27	-	-	0.89	-	0.77	0.77
Leucine	0.96	0.96	-	-	1.56	-	1.69	1.66
Lysine	0.28	0.26	-	-	1.29	-	1.01	0.99
Methionine	0.22	0.23	-	-	0.34	-	0.37	0.36
Phenylalanine	0.41	0.40	-	-	1.01	-	0.92	0.91
Threonine	0.26	0.25	-	-	2.30	-	0.63	0.61
Valine	0.43	0.39	-	-	0.95	-	0.90	0.90

continued

Table 3.3. continued

Dispensable amino acid								
Alanine	0.61	0.59	-	-	0.87	-	0.98	0.97
Aspartate	0.57	0.56	-	-	2.30	-	1.85	1.81
Cysteine	0.15	0.15	-	-	0.22	-	0.25	0.24
Glutamate	1.54	1.50	-	-	3.74	-	3.42	3.36
Glycine	0.34	0.32	-	-	0.86	-	0.78	0.77
Proline	0.74	0.70	-	-	1.01	-	1.10	1.07
Serine	0.45	0.45	-	-	1.19	-	1.06	1.01
Tyrosine	0.34	0.34	-	-	0.71	-	0.69	0.68

[a] LPC = low phytate corn; NC = normal corn; LPC-iCa-iP = low phytate corn, limestone, and monosodium phosphate; NC-iCa-iP = normal corn, limestone, and monosodium phosphate; SBM = soybean meal; SBM-iCa-iP = soybean meal, limestone, and monosodium phosphate; LPC-SBM-iCa-iP = low phytate corn, soybean meal, limestone, and monosodium phosphate; NC-SBM-iCa-iP = normal corn, soybean meal, limestone, and monosodium phosphate

Table 3.4. Main use of diets and method of determinations[a]

Diet	Ingredients	Method	Determinations
1	LPC	Direct	AID of CP and AA in LPC
		Direct[b]	AID and AFD of Ca and P in LPC
2	NC	Direct	AID of CP and AA in NC
		Direct[b]	AID and AFD of Ca and P in NC
3	LPC, iCa, iP	Direct	AID and AFD of Ca and P in diet
4	NC, iCa, iP	Direct	AID and AFD of Ca and P in diet
5	SBM	Direct	AID of CP and AA in SBM
		Direct	AID and AFD of Ca and P in SBM
6	SBM, iCa, iP	Direct	AID and AFD of Ca and P in diet
7	LPC, SBM, iCa, iP	Direct	AID of CP and AA in diet
		Difference	AID of CP and AA in LPC
		Direct	AID and AFD of Ca and P in diet
8	NC, SBM, iCa, iP	Direct	AID of CP and AA in diet
		Difference	AID of CP and AA in NC
		Direct	AID and AFD of Ca and P in diet
9	Protein free diet	Direct	Endogenous losses of CP and AA

continued

Table 3.4 continued

[a] LPC = low phytate corn, NC = normal corn, iCa = limestone, iP = monosodium phosphate, SBM = soybean meal, AID = apparent ileal digestibility coefficients, AFD = apparent fecal digestibility coefficients

[b] The AID and AFD of Ca for diets 1 and 2 were calculated as W = [A – (X x Y)]/Z where W=the AID (%) of Ca in LPC or NC, A=the AID (%) of either LPC-iCa-iP or the NC-iCa-iP diet, X=the AID (%) of iCa (determined by difference method), Y=the amount of iCa (decimal %) in either the LPC-iCa-iP or the NC-iCa-iP diet, Z=the amount of either the LPC or NC (decimal %) in either the LPC-iCa-iP or the NC-iCa-iP diet. It should also be noted that A=AID of iCa was calculated using the difference method, which is = [apparent digestibility of the SBM-iCa diet – (apparent digestibility of Ca in the SBM diet x relative contribution of the SBM to total composition Ca in the SBM-iCa diet)]/relative contribution iCa to total composition of Ca in the SBM-iCa

Table 3.5. Apparent digestibility coefficients (%) of calcium and phosphorus in low phytate corn, normal corn, soybean meal, and corn-soybean meal diets [abc]

	Dietary treatment								
Treatment No.:	1	2	3	4	5	6	7	8	
Diet description	LPC	NC	LPC-iCa-iP	NC-iCa-iP	SBM	SBM-iCa-iP	LPC-SBM-iCa-iP	NC-SBM-iCa-iP	SEM[d]
Item									
Calcium									
AID [efg]	70.04[v]	47.37[wx]	63.42[vy]	42.98[w]	50.91[wz]	57.16[yz]	55.01[xyz]	51.04[wz]	4.87
AFD [efg]	69.06[w]	49.55[xy]	63.21[wx]	45.93[yz]	46.73[yz]	50.10[xz]	-	-	7.63
Phosphorus									
AID [ef]	56.50[w]	28.34[x]	49.68[wy]	44.91[y]	37.16[z]	49.56[wy]	47.93[y]	49.83[wy]	3.66
AFD[ef]	54.47[w]	28.84[x]	50.71[wz]	49.75[wz]	38.04[y]	46.23[z]	-	-	3.40

continued

Table 3.5. continued

[a] AID and AFD were calculated as 100 – [(intake – excreted)/intake] x 100%

[b] Values are least square means for nine pigs per treatment

[c] LPC = low phytate corn; NC = normal corn; LPC-iCa-iP = low phytate corn, limestone, and monosodium phosphate; NC-iCa-iP = normal corn, limestone, and monosodium phosphate; SBM = soybean meal; SBM-iCa-iP = soybean meal, limestone, and monosodium phosphate; LPC-SBM-iCa-iP = low phytate corn, soybean meal, limestone, and monosodium phosphate; NC-SBM-iCa-iP = normal corn, soybean meal, limestone, and monosodium

[d] Pooled standard error of the mean

[e] AID = apparent ileal digestibility coefficients (%); AFD = apparent fecal digestibility coefficients (%)

[f] No differences ($P > 0.10$) between ileal and fecal Ca, or ileal and fecal P

[g] The AID and AFD of Ca for diets 1 and 2 were calculated as W = [A – (X x Y)]/Z where W=the AID (%) of Ca in LPC or NC, A=the AID (%) of either LPC-iCa-iP or the NC-iCa-iP diet, X=the AID (%) of iCa (determined by difference method), Y=the amount of iCa (decimal %) in either the LPC-iCa-iP or the NC-iCa-iP diet, Z=the amount of either the LPC or NC (decimal %) in either the LPC-iCa-iP or the NC-iCa-iP diet. It should also be noted that A=AID of iCa was calculated using the difference method, which is = [apparent digestibility of the SBM-iCa diet – (apparent digestibility of Ca in the SBM diet x relative contribution of the SBM to total composition Ca in the SBM-iCa diet)]/relative contribution iCa to total composition of Ca in the SBM-iCa where D_A is the AID (%) of AA in the LPC or NC, D_D is the AID (%) in either the LPC-SBM or the NC-SBM diet measured by the direct method, D_B is the AID (%) of AA in SBM measured by the direct method, S_B is the contribution level of an AA (decimal %) from the SBM in the LPC-SBM and NC-SBM diets, and S_A is the contribution level of that AA (decimal %) from either the LPC or NC in the corn-SBM diets.

[vwxyz] means within a row lacking a common superscript are different ($P < 0.05$)

Table 3.6. Apparent ileal digestibility coefficients (%) of crude protein and amino acids in low phytate corn, normal corn, soybean meal, and corn-soybean meal diets calculated using the direct method [abc]

	Dietary treatments					
Treatment No.:	1	2	5	7	8	
Diet description	LPC	NC	SBM	LPC-SBM	NC-SBM	SEM[d]
Item						
Crude protein	68.19 [y]	64.46 [y]	78.56 [z]	78.04 [z]	78.75 [z]	2.00
Indispensable amino acids						
Arginine	79.59 [w]	75.12 [x]	92.09 [y]	88.35 [z]	88.82 [z]	1.28
Histidine	78.41 [x]	76.57 [x]	87.88 [y]	83.52 [z]	84.53 [z]	1.08
Isoleucine	71.19 [w]	67.93 [x]	83.22 [y]	77.98 [z]	79.47 [z]	1.49
Leucine	84.48 [y]	83.29 [yz]	84.16 [y]	81.80 [z]	82.99 [yz]	0.99
Lysine	65.51 [w]	57.43 [x]	86.73 [y]	81.42 [z]	82.24 [yz]	2.38
Methionine	79.72	79.53	80.78	77.79	78.11	1.70
Phenylalanine	80.77 [x]	77.75 [y]	85.11 [z]	81.50 [x]	82.47 [xz]	1.38
Threonine	61.82 [w]	57.03 [x]	76.75 [y]	70.49 [z]	71.89 [z]	1.91
Valine	67.36 [w]	61.55 [x]	75.32 [y]	70.83 [wz]	73.36 [yz]	1.97

continued

Table 3.6. continued

Dispensable amino acids						
Alanine	76.91 [w]	74.10 [wy]	75.58 [wz]	73.23 [xyz]	76.21 [wz]	1.55
Aspartate	69.23 [w]	65.77 [x]	83.35 [y]	78.26 [z]	79.41 [z]	1.45
Cysteine	75.10	75.23	75.12	73.20	74.21	2.02
Glutamate	81.61 [wx]	80.63 [w]	86.35 [y]	83.62 [xz]	84.60 [yz]	1.13
Glycine	44.73 [x]	34.92 [y]	68.51 [z]	62.60 [z]	64.41 [z]	3.73
Proline	32.37 [y]	28.12 [y]	68.45 [z]	61.07 [z]	63.34 [z]	9.97
Serine	71.54 [x]	68.99 [x]	81.64 [y]	75.78 [z]	76.93 [z]	1.57
Tyrosine	79.77 [xy]	77.92 [y]	84.42 [z]	80.46 [x]	82.04 [xz]	1.17

[a] AID was calculated as [(intake – excreted)/intake] x 100%

[b]Values are least square means for nine pigs per treatment

[c] LPC = low phytate corn; NC = normal corn; SBM = soybean meal; LPC-SBM = low phytate corn, soybean meal, limestone, and monosodium phosphate; NC-SBM = normal corn, soybean meal, limestone, and monosodium

[d] Pooled standard error of the mean

[wxyz] means within a row lacking a common superscript are different ($P < 0.05$)

Table 3.7. Standardized ileal digestibility coefficients (%) of crude protein and amino acids in low phytate corn, normal corn, soybean meal, and corn-soybean meal diets calculated using the direct method [abc]

	Dietary treatments					
Treatment No.:	1	2	5	7	8	
Diet description	LPC	NC	SBM	LPC-SBM	NC-SBM	SEM[d]
Item						
Crude protein	87.92	84.84	87.54	85.82	87.10	1.98
Indispensable amino acid						
Arginine	92.88 ^{xy}	89.97 ^{x}	96.24 ^{z}	93.23 ^{yz}	93.74 ^{yz}	1.49
Histidine	85.89 ^{wx}	84.63 ^{w}	91.48 ^{y}	87.18 ^{xz}	88.31 ^{z}	1.07
Isoleucine	85.95 ^{yz}	83.38 ^{y}	87.97 ^{z}	83.50 ^{y}	85.02 ^{yz}	1.48
Leucine	89.90 ^{w}	88.73 ^{wx}	87.52 ^{xy}	84.90 ^{z}	86.15 ^{yz}	0.98
Lysine	81.31 ^{x}	74.37 ^{y}	90.19 ^{z}	85.84 ^{xz}	86.76 ^{z}	2.49
Methionine	87.72 ^{y}	87.26 ^{y}	86.05 ^{yz}	82.64 ^{z}	83.11 ^{z}	1.79
Phenylalanine	88.53 ^{x}	85.68 ^{yz}	88.23 ^{xy}	84.91 ^{z}	85.95 ^{xyz}	1.37
Threonine	80.45 ^{xz}	76.23 ^{y}	83.62 ^{z}	78.19 ^{xy}	79.85 ^{xyz}	1.90
Valine	85.36 ^{y}	81.38 ^{yz}	83.41 ^{yz}	79.41 ^{z}	81.94 ^{yz}	2.07

continued

Table 3.7. continued

Dispensable amino acids						
Alanine	88.16 ^{x}	85.57 ^{xz}	83.39 ^{yz}	80.19 ^{y}	83.23 ^{yz}	1.57
Aspartate	85.26 ^{xy}	82.21 ^{x}	87.33 ^{yz}	83.20 ^{x}	84.47 ^{xz}	1.51
Cysteine	85.21 ^{y}	85.66 ^{y}	82.18 ^{yz}	79.41 ^{z}	80.73 ^{z}	2.05
Glutamate	89.17 ^{yz}	88.36 ^{yz}	89.45 ^{y}	87.01 ^{z}	88.06 ^{yz}	1.14
Glycine	88.56 ^{x}	82.18 ^{x}	85.76 ^{x}	81.77 ^{x}	83.67 ^{x}	3.67
Proline	106.57 ^{x}	105.64 ^{x}	122.72 ^{x}	110.55 ^{x}	114.28 ^{x}	9.98
Serine	87.51 ^{y}	84.71 ^{yz}	87.63 ^{y}	82.51 ^{z}	83.97 ^{z}	1.54
Tyrosine	88.71 ^{y}	87.05 ^{yz}	88.76 ^{y}	84.89 ^{z}	86.57 ^{yz}	1.17

[a] SID was calculated as {[intake – (excreted – endogenous)]/intake} x 100%

[b] Values are least square means for nine pigs per treatment

[c] LPC = low phytate corn; NC = normal corn; SBM = soybean meal; LPC-SBM = low phytate corn, soybean meal, limestone, and monosodium phosphate; NC-SBM = normal corn, soybean meal, limestone, and monosodium

[d] Pooled standard error of the mean

[wxyz] means within a row lacking a common superscript are different ($P < 0.05$)

Table 3.8. Endogenous losses (mg/kg DMI) of protein and amino acids in growing pigs [ab]

Item	Endogenous amino acid losses
Crude Protein	17,659
Indispensable amino acids	
Arginine	590
Histidine	188
Isoleucine	425
Leucine	524
Lysine	447
Methionine	179
Phenylalanine	315
Threonine	485
Valine	769

continued

Table 3.8. continued

Dispensable amino acids	
Alanine	683
Aspartate	915
Cysteine	156
Glutamate	1,160
Glycine	1,492
Proline	5,459
Serine	711
Tyrosine	307

[a] Values are the least square mean of nine pigs

[b] Calculated as EAL=AAd x (Crf/Crd), where EAL=endogenous AA loss, AAd=AA in the digesta, Crf=chromium in the feed, Crd=chromium in the digesta

Table 3.9. Apparent ileal digestibility coefficients (%) of crude protein and amino acids in low phytate corn and normal corn calculated using the difference method [abc]

	Corn types		
Item	LPC [d]	NC [d]	SEM[e]
Crude protein	77.40	79.03	4.68
Indispensable amino acids			
Arginine	79.39	80.70	3.62
Histidine	77.51	79.55	2.60
Isoleucine	67.91	72.15	5.02
Leucine	79.17	81.65	2.51
Lysine	64.66	66.58	6.38
Methionine	72.67	74.74	4.42
Phenylalanine	75.27	77.69	3.26
Threonine	58.58	61.65	6.61
Valine	63.79	70.29	4.92

continued

Table 3.9. continued

Dispensable amino acids			
Alanine	70.72	76.90	4.23
Aspartate	63.52	66.92	6.55
Cysteine	70.88	73.00	6.64
Glutamate	78.39	81.06	4.00
Glycine	51.40	56.56	10.12
Proline	51.25	56.07	22.76
Serine	63.74	65.72	5.96
Tyrosine	74.40	78.21	3.73

[a] AID calculated as {[digestibility of both corn-SBM diets – (digestibility of the SBM diet x relative contribution of the SBM to total composition of the AA in the corn-SBM diets)] /relative contribution of both corns to total composition of the AA in the corn-SBM diets}

[b] Values are least square means for nine pigs per treatment

[c] LPC = low phytate corn, NC = normal corn

[d] No differences (*P* > 0.15) in apparent ileal digestibility coefficients of CP or any AA in LPC and NC using the difference method

[e] Pooled standard error of the mean