Phosphorus Digestibility of Inorganic Phosphorus Sources by Growing Pigs

By

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Phosphorus Digestibility of Inorganic Phosphorus Sources by Growing Pigs

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

Phosphorus digestibility of inorganic phosphorus sources by growing pigs

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A series of studies was conducted with the objective of formulating, implementing, and verifying a new method for measuring phosphorus (P) digestibility in feed ingredients. The first study was designed to measure amino acid digestibility in a newly developed diet. Apparent (AID) and standardized (SID) ileal digestibility were measured in soybean meal (SBM) and in two P-free diets based on pork-gelatin or beef-gelatin. Results showed that the two gelatin sources were similar to SBM in the digestibility of all amino acids except for His and Thr in the beef gelatin diet. With the supplementation of crystalline His, Ile, Met, and Trp, both gelatin sources were found to be well suited as amino acid sources in a P-free diet. In the second study, the P-free diet based on pork-gelatin was used to measure apparent (ATTD) and true (TTTD) total tract digestibility of P in five different sources of inorganic P. The five feed phosphates were dicalcium phosphate (DCP), monocalcium phosphate with 70% purity (MCP70), monocalcium phosphate with 85% purity (MCP85), monocalcium phosphate with 100% purity (MCP100), and monosodium phosphate (MSP). The ATTD of P for MSP was higher ($P < 0.05$) than for DCP, MCP70, and MCP85 (91.9 vs. 81.5, 82.6, and 81.7%, respectively), but the ATTD for MCP100 (88%) was not different from any of the other

P-sources. The endogenous loss of P was measured in pigs fed the P-free basal diet and was estimated at 0.139 g per kg DMI. For MSP, the TTTD was 98.2%. This value was higher ($P < 0.05$) than the TTTD for DCP, MCP70, and MCP 85 (88.2, 89.5, and 88.4%, respectively). For MCP100, a TTTD of 94.9% was calculated – this number was not different from the TTTD for any of the other P-sources. In the final study, the same P-sources as used in exp. 2 were used in a slope-ratio assay to measure the relative bioavailability of P in each source, and these values were compared to the values for bone density. It was found that MSP, MCP100, MCP85, and MCP70 had a higher ($P < 0.05$) bone ash contents than DCP. Likewise, pigs fed MSP had a higher bone breaking strength than pigs fed DCP. Global bone mineral content and global bone mineral density were measured on Dual-energy X-ray Absorptiometry (DXA). The cortical area and total mineral content of bone at the 50% slice was measured on Peripheral Quantitative Computed Tomography (pQCT), and had a high correlation with bone breaking strength. It was concluded that P in MSP has the highest digestibility and availability of the inorganic feed phosphates, followed by MCP100. Numerically, the lowest digestibility and availability of P is in DCP, while there are no differences between the different sources of MCP.

Key words: Phosphorus, Phosphorus digestibility, Phosphorus-free diet, Pig, Relative bioavailability, Bone density

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LIST OF ABBREVIATIONS

AA	Amino acid
AAd	Amino acid content of dry matter ileal digesta
AAf	Amino acid content of the dry matter feed
ADFI	Average daily feed intake
ADG	Average daily gain
AID	Apparent Ileal Digestibility coefficient(s)
Al	Aluminum
Ala	Alanine
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
Arg	Arginine
Asp	Aspartic acid
ATTD	Apparent Total Tract Digestibility coefficient(s)
BMC	Bone mineral content
BMD	Bone mineral density
BW	Body Weight
ºC	Degrees Celsius

Ca	Calcium
cAMP	Cyclic adenosine monophosphate
cm	Centimeters
CP	Crude Protein
Crd	Chromium content of dry matter in ileal digesta
Crf	Chromium content of the dry matter in feed
Cu	Copper
Cys	Cysteine
d	Day
DCP	Dicalcium phosphate
DM	Dry Matter
DMI	Dry matter intake
DXA	Dual-energy X-ray absorptiometry
EAL	Endogenous amino acid losses
EPL	Endogenous phosphorus losses
Fe	Iron
g	Grams
G:F	Gain to feed ratio

Glu	Glutamine
Gly	Glycine
H	Hydrogen
H	Hours
HCl	Hydrochloric acid
His	Histidine
HPLC	High performance liquid chromatography
I	Iodine
Ile	Isoleucine
IU	International units
Kcal	Kilocalories
kg	Kilograms
Leu	Leucine
Lys	Lysine
Mcal	Megacalories
MCP70	Monocalcium phosphate with 70% purity
MCP85	Monocalcium phosphate with 85% purity
MCP100	Monocalcium phosphate with 100% purity

ME	Metabolizable energy
Met	Methionine
Mg	Magnesium
mg	Milligrams
Mn	Manganese
MSP	Monosodium phosphate
N	Nitrogen
Na	Sodium
NRC	National research council
O	Oxygen
P	Phosphorus
Pf	Fecal output of phosphorus
Phe	Phenylalanine
Pi	Phosphorus intake
pQCT	Peripheral quantitative computed tomography
Pr	Phosphorus retention
Pro	Proline
PTH	Parathyroid hormone

Pu	Phosphorus content in the urine
R1	Region 1 of dual-energy X-ray absorptiometry: 0-20% of the tibia from the distal end
R2	Region 2 dual-energy X-ray absorptiometry: 20-40% of the tibia from the distal end
R3	Region 3 dual-energy X-ray absorptiometry: 40-60% of the tibia from the distal end
R4	Region 4 dual-energy X-ray absorptiometry: 60-80% of the tibia from the distal end
R5	Region 5 dual-energy X-ray absorptiometry: 80-100% of the tibia from the distal end
SAA	Sulfur containing amino acids
SAS	Statistical analysis software
SBM	Soybean meal
SDSU	South Dakota State University
Se	Selenium
SEM	Standard error of the mean
Ser	Serine
SID	Standardized ileal digestibility coefficient(s)

Thr	Threonine
SSI	Strength strain indices
Trp	Tryptophan
TTTD	True total tract digestibility
Tyr	Tyrosine
U.S.	United States of America
UV	Ultra-violet
Val	Valine
wk	Week
Zn	Zinc

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CHAPTER 1

Introduction

The United States government has declared phosphorus (P) to be a major cause of environmental pollution in agriculture. Because of this declaration, livestock producers need a better measure of P digestibility to predict the excretion of P from livestock. Historically, diets for swine have been formulated on the basis of total P concentration although it is recognized that P in feed ingredients is not completely digested and absorbed. It is also recognized that there are differences in the digestibility of P in different sources occur. However, there is no universally accepted method for estimating the digestibility of P in animals that are fed different feed ingredients. The most common measure of P-utilization is measures of relative bioavailability of P in different feed ingredients. Relative bioavailability coefficients obtained from the slope-ratio technique express the availability P in one P-source relative to the availability of P in a standard, which is often, but not always, monosodium phosphate (MSP). The availability of MSP is assumed to be 100%. Two possible problems exist. Monosodium phosphate may or may not be 100% available and the values obtained can not be compared to other studies where different standards are used. In addition, the excretion of P from the animals is not estimated using this procedure. To obtain values for P-utilization from feed ingredients, which can be compared between studies and is globally acceptable, a new procedure needs to be developed. The implementation of this procedure should determine both P-absorption from feed ingredients and the excretion of P from animals fed these ingredients. The procedure should be applicable to all feed ingredients and allow for the

comparison of results from different studies. In addition, the values obtained for individual feed ingredients need to be additive in a mixed diet.

If such a procedure can be developed, the swine industry would be better able to minimize the excretion of P from animals.

CHAPTER 2

Phosphorus digestibility of inorganic phosphorus sources by growing pigs: Literature review

1. Introduction

Phosphorus (P) has become a regulated nutrient. Government regulations have dictated a reduction of P application to agricultural lands, which will increase the number of acres required to apply manure. To aid in this reduction, producers are looking for options to reduce excretion of P from livestock. To predict excretion, producers need to know how much P will remain in the body of an animal when fed a particular feed. To date, in the swine industry, only a rough estimate of P-digestibility in feed ingredients is available to producers for use in feed formulation.

However, to reduce P-pollution, producers need to feed highly digestible P-sources and avoid feeding more digestible P than what the pigs require for maximum productivity. Therefore, digestibility for P in various feed ingredients is needed. By using digestibility coefficients, P-sources with a high digestibility can be selected and the inclusion of P in diets for swine can be limited to only what is needed by the animals.

To satisfy growing pigs' requirement for phosphorus, it is usually necessary to include a source of inorganic P in the diets fed to growing pigs in addition to the organic P present in the other feed ingredients (Jongbloed et al., 1991). Because organic P in plants is bound to a phytate complex, inorganic P usually has a higher digestibility coefficient for pigs than has organic P (Jongbloed et al., 1991). Monocalcium phosphate

(MCP) or dicalcium phosphate (DCP) is often used as an inorganic source of P. It has been shown that MCP is more digestible to pigs than is DCP (Huyghebaert et al., 1980; Jongbloed, 1987), but differences between sources of MCP exist, because in reality all MCP sources are mixtures of MCP and DCP (Jongbloed et al., 1991). Monocalcium phosphate actually contains between 40% and 85% MCP. It is assumed that the more MCP a source contains, the higher digestibility that source will have. Additionally, if water is attached to the P molecule, it has a higher availability than if no water is attached (Grimbergen et al., 1985). Therefore, there may be differences in the digestibility between different MCP sources.

2. Digestion and absorption of dietary phosphorus

Organic P is naturally bound in the phytate molecule. The bonds between the P and the inositol molecule in phytate need to be digested before the P becomes available to the animal. Intestinal phosphatases, such as alkaline phosphatase and intestinal phytase, break the phytate bonds and release inorganic P at the brush border of the enterocytes. Only inorganic P is readily absorbed by the small intestine. However, pigs do not secrete phytase in their gastrointestinal tracts, therefore, P bound in phytate is not digested by pigs, and organic P has a low digestibility. On the other hand, P from inorganic sources (i. e., DCP and MCP) is much more digestible.

2.1 Mechanism of phosphorus absorption

Phosphorus is absorbed as inorganic P in the proximal half of the small intestine (Jongbloed, 1987). Little or no P absorption occurs in the large intestine; therefore, any

undigested P reaching the distal ileum is excreted in the feces (Liesegang et al., 2002). Both passive non-saturable transport and secondary Na^+- coupled active transport mechanisms are present in the small intestine (Breves and Schroder, 1991). Diffusion of P occurs across the epithelial cells lining the lumen of the intestines, down the concentration gradient, until equilibrium is reached. The Na^+- coupled active transport symporters can pump P against the concentration gradient into the cytosol of the enterocyte. First, the P is transported across the brush border membrane into the enterocyte using a secondary active Na^+ - inorganic P cotransport system. Next, the P is transported to the basolateral side of the cell, and finally the P is transported across the basolateral membrane; however, these mechanisms are not fully understood (Breves and Schroder, 1991). Phosphorus is absorbed and transported through the blood as inorganic P, or as a part of phospholipids.

2.2 Regulation of phosphorus homeostasis

Parathyroid hormone (PTH), calcitonin, alkaline phosphatase, and vitamin D help regulate P absorption and metabolism. Parathyroid hormone is secreted from the parathyroid gland and secretion is inversely dependant on the concentration of serum Ca (Jongbloed, 1987). The main effects of PTH on P metabolism are through kidney function and bone resorption. Parathyroid hormone stimulates the bone to release P into the P pool. The P can then be secreted into urine in the kidneys. When in the kidneys, PTH stimulates an increase in cAMP production in the proximal tubule, the release of P into the blood, and the excretion of P through the urine (Stanton and Koeppen, 2004). Calcitonin stimulates bone formation and increased renal excretion of P. Secretion of

calcitonin from the thyroid gland is stimulated by hypercalcemia (Stanton and Koeppen, 2004). Alkaline phosphatase hydrolyzes phosphate ester bonds improving absorption across the brush border (Jongbloed, 1987). Vitamin D stimulates P absorption in the small intestine. Provitamin D from the diet is present in the intestines, absorbed, and transported to the skin. Alternatively, provitamin D is synthesized in the body from cholesterol. Ultraviolet light reacts with provitamin D to form previtamin D. Previtamin D is converted to 1, 25-$(OH)_2D_3$ or calcitriol, which is the active form of vitamin D (Jongbloed, 1987). Calcitriol will stimulate Ca and P absorption in the intestine at the mucosal border, release Ca and P from the bone, and decrease Ca and P excretion from the kidneys. Calcitriol production is stimulated by hypocalcemia via PTH and hypophosphatemia (Stanton and Koeppen, 2004). Excessive intakes of Ca, Fe, Al and Mg may form insoluble mineral phosphates and may decrease P absorption (McDowell, 1992). For this reason, the dietary Ca:P ratio should be maintained between 1:1and 2:1.

3. Phosphorus digestibility and availability

3.1 Digestibility vs. availability

Digestibility values are measured by determining the difference between P-intake and fecal excretion (Jongbloed et al., 1991). Two main methods are used to make this determination (i.e., the difference method and the direct method). In the difference method, one source of P is fed and P-digestibility is measured. This is called the basal diet. Then this source is mixed in a diet with a second source of P (the test ingredient). The mixture is fed and P-digestibility is determined for this diet. By subtracting the

amount of digestible P provided by the basal diet from the total amount of digestible P in the mixture, the digestible amount of P originating from the test feed ingredient can be calculated. By expressing this as a percentage of total P in the test feed, the P-digestibility for this source is calculated. The direct method consists of a diet in which there is only one P-containing ingredient (the test ingredient); all other ingredients in the diet contain no P. The digestibility of P in the diet equals the digestibility of the P in the test feed ingredient.

Relative bioavailability is measured by comparing a highly digestible P-source (standard P-source) to an unknown source using a slope-ratio procedure (Cromwell, 1992). There is a basal diet, two or three levels of test diets, and two or three levels of the standard diet containing the highly digestible P-source. The basal diet has a low P-content, and is used to determine the common starting point. The test P-sources are added to the basal diet at two or three graded levels in such a way that all diets have a P-concentration that is below the pigs' requirement for P. Likewise, the standard diets consist of the basal diet plus the standard P-source at two or three levels of P fed below the pigs' requirement for P. All diets are fed for a period of four to six weeks. At the conclusion of the experiment, pigs are killed and one or more bones are harvested, and the bone ash or bone breaking strength is determined. Bone ash or breaking strength is then regressed on P-intake for each source of P. The slope of the regression line for each P-source is determined and entered into the following equation: relative bioavailability = 100(slope B/ slope A). Slope B is the regression slope for the test diets, and slope A is the regression slope for the standard diets (Cromwell, 1992). Therefore, the availability

of P in the test ingredient is expressed relative to the availability of the highly digestible P-source in the standard diets.

The main difference between the slope-ratio procedure and the digestibility methods is how the availability of the P-source is expressed. The digestibility procedures report a digestibility value for P in the test ingredient. The slope-ratio assays report the relative bioavailability of P. This means that one source of P is expressed as a percentage of another standard source. The standard source may or may not be 100% digestible. Therefore, the relative bioavailability values cannot be compared between studies if different P-sources were used in the standard diets. In addition, the digestibility procedures allow for the estimation of the quantity of P excreted in the feces from the pigs, which cannot be calculated from data, obtained using the slope-ratio procedure.

3.2 Apparent and true total tract digestibility

Because availability coefficients are based off of a source that may or may not be 100% digestible, more accurate results may be obtained by measuring apparent and true digestibility coefficients for P. This technique requires the estimation of endogenous losses of P. This can be accomplished by formulating a P-free diet, but such a diet has never been successfully formulated. However, if a P-free protein source is identified, it may be possible to formulate such a diet. By feeding the P-free source, the endogenous P can be directly determined by measuring the total P-output. The P-free diet may also be used to directly measure P-digestibility of inorganic sources of P by adding these sources to the P-free diet. Apparent total tract digestibility (ATTD) is defined as the amount of P taken in by the animal minus the amount that is excreted in total fecal output; this is then

divided by the P intake. To obtain true total tract digestibility (TTTD) the endogenous losses, estimated after feeding the P-free diet, is subtracted from the output of P and then divided by dietary P-intake. It is believed that digestibility coefficients based on TTTD are more additive in a mixed ration than are values based on ATTD (Fan et al., 2001). Therefore, TTTD for P in various feed ingredients, such as inorganic P-sources, are needed.

3.3 Endogenous phosphorus losses

Endogenous losses are P excreted from the body that is not of dietary origin. Previous estimates for endogenous losses have been 90-630 mg/kg DMI (Ajakaiye et al., 2003); 570-840 mg/kg DMI (Shen et al., 2002); 140-320 mg/kg DMI (Fan et al., 2001); 70 mg/kg DMI (Pettey et al., 2003); and 70-800 mg/kg DMI (Jongbloed, 1987). All previous data on endogenous losses were obtained by regressing several levels of P back to zero P-intake. The reason this approach has been used is that so far, a P-free diet has never been formulated.

4. Bone measurements

4.1 Bone breaking strength and bone ash studies

Bone breaking strength is a common method for the determination of P availability. As bone mineralization increases, maximum stress and bending moment of the bone will increase (Crenshaw et al., 1981). If the P-source is more available, it will deposit more mineral in the bone, increasing the breaking strength. In these studies, the third and fourth metacarpals are commonly used to measure breaking strength. The soft

tissue is removed from the bones. The metacarpals are then placed in the bone breaking instrument, and the breaking strength is recorded. Bone ash can also be determined in these bones. After breaking, the bones are defatted to remove any excess organic matter. The bones are weighed, placed in a muffle furnace for 16 h at 600˚C, and weighed again after ashing. The values from the bone breaking strength or bone ash are then plotted in a regression line, and bioavailability is determined using the slope ratio technique. This technique is used to measure relative P-availability for many feed ingredients. Dicalcium phosphate has a relative bioavailability of 95-100% (NRC, 1998). Others have shown DCP availability to be 102-107% (Cromwell, 1989), 81-105% (Nelson et al., 1990), 63-69% (Jongbloed et al., 1991), and 52-73% (Eekhout and De Paepe, 1996) relative to monosodium phosphate (MSP). Grimbergen et al. (1985) reported a relative availability of anhydrated DCP at 88% using hydrate DCP as the standard. Rodehutscord et al. (1994) used the difference method to report a digestibility of DCP at 87%.

The NRC (1998) indicates MCP to have a relative availability of 100%. Researchers have demonstrated availability values of MCP at 100-102% (Nelson et al., 1990), 71-84% (Jongbloed et al., 1991), and 64-92% (Eekhout and De Paepe, 1996) relative to MSP. Grimbergen et al. (1985) reported a relative availability of MCP to be 120% using hydrated DCP as the standard. Rodehutscord et al. (1994) used the difference method to report the digestibility of MCP to be 91%.

When using the slope ratio technique MSP is often used as the reference and the availability of P in MSP is assumed to be 100% (Jongbloed, 1987). National Research Council (1998) also states the relative bioavailability of MSP to be 100%. Rodehutscord

et al. (1994) reported the availability of MSP to be 96% when using the difference method.

4.2 Bone density studies

Bone density is another option for the determination of P availability. Density measurements can be obtained in a live animal. Therefore, many observations can be made on the same animal over time, resulting in a more accurate analysis. Densitometers have been used for determination of bone mineral, fat, lean content and total tissue mass (Mitchell et al., 1998). Furthermore, Dual-energy X-ray Absorptiometry (DXA) and Peripheral quantitative computed tomography (pQCT) can be used to measure bone density in clinical trials (Binkley and Specker, 2000). Bone mineral density (BMD) and bone mineral content (BMC) are the most widely used parameters obtained by DXA. The method of choice for evaluating bone mineral status is DXA (Binkley and Specker, 2000). Some criticize the BMD measurement because it is influenced by bone area (Lochmüller et al., 2000, Binkley and Specker, 2000).

It has been argued that the use of pQCT is a better measure of bone density. This is because pQCT uses volume in its measure of BMD rather than area (Binkley and Specker, 2000). Crenshaw et al. (1981) indicates the importance of correct calculation of bone breaking strength. These researchers also indicate the moment of inertia as an important consideration when making these calculations. Schiessl et al. (1996) also indicates the importance of the moment of inertia on bone breaking strength. Crenshaw et al. (1981) demonstrated that the modulus of elasticity is an important consideration. The modulus of elasticity is a measure of the capacity of the bone to return to its original

shape after it has been deformed by force (Crenshaw et al., 1981). Schiessl et al. (1996) stated that the moment of inertia and modulus of elasticity are directly proportional to bone strength. The moment of inertia can be measured by pQCT. However, the modulus of elasticity cannot be measured, but volumetric cortical density, which is closely related to the modulus of elasticity, can be measured by pQCT (Schiessl et al., 1996). By using the moment of inertia and volumetric cortical density, the strength strain index (SSI) can be derived. This calculation can be used to predict bone strength with respect to bending and torsion (Schiessl et al., 1996).

4.3 Correlation of bone density to bone breaking strength

Lochmüller et al. (2000) demonstrated that there is no difference between ash weight and BMC. Schiessl et al. (1996) found a high correlation between noninvasive SSI and muscle force, using the pQCT method for the estimation of bone strength. It may be possible to use DXA or pQCT to measure the mineralization of bone.

5. Conclusion and perspectives

There is a need for measuring TTTD values for inorganic and organic P-sources, because TTTD values are additive in mixed diets. These values are needed to formulate diets that match the pigs' requirement of P and to reduce P-excretion. To obtain TTTD, a way of accurately estimating endogenous P losses needs to be developed. If a P-free diet can be developed, this diet may be used to not only measure endogenous losses of P, but also be used as the basis for measuring ATTD and TTTD in inorganic P-sources.

The slope ratio technique requires two to three graded levels of P, but using the P-free diet, ATTD and TTTD can be measured using only one diet per P-source. The P-free diet method, however, has yet to be developed and validated.

The values for ATTD and TTTD may be different from the values obtained using the slope-ratio technique, but the ranking of individual P-sources should be similar.

An alternative to measuring bone breaking strength is to estimate bone density by scanning. However, these values also need to be compared to bone breaking strength to be validated. Also, because it is possible to obtain multiple bone measurements using pQCT or DXA scanning, it is necessary to identify which measurements are most closely correlated to bone breaking strength

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CHAPTER 3

Apparent and standardized ileal amino acid digestibility coefficients of amino acid-fortified cornstarch-based gelatin-supplemented diets by growing pigs

ABSTRACT: A study was conducted with the objective of measuring apparent (AID) and standardized (SID) ileal digestibility coefficients of amino acids (AA) in two gelatin-containing diets and in soybean meal (SBM) by growing pigs. Four individually housed pigs (BW: 44.05 ± 1.45 kg) were equipped with a simple T-cannula in the distal ileum. Digesta were collected from these pigs in 7-d periods. Two diets using gelatin of pork or beef origin, were formulated using mainly cornstarch, sucrose, gelatin, and crystalline DL-methionine, L-tryptophan, L-isoleucine, and L-histidine. Two additional diets were formulated, one included SBM, and the other was an N-free diet. The N-free diet was used to estimate the basal endogenous losses of CP and AA. Results showed that there were no differences in AID and SID for any of the indispensable AA between the pork gelatin-containing diet and SBM. The beef gelatin based diet had a lower AID for His and Thr, and a lower SID for Thr compared to SBM. The gelatin sources are similar in AID and SID to SBM, and therefore with supplementation of His, Ile, Met, and Trp, gelatin sources are well suited for inclusion in a P-free diet.

Key Words: Amino acid, Digestibility, Gelatin, Pig, P-free diet

Introduction

The excretion of phosphorus from swine has come under scrutiny during recent years because excretion is considered a major part of P pollution. To reduce this pollution, several U. S. states have implemented a manure handling system based on the amount of P that is present in manure. It has determined that the more P present in manure, the more acres are required for spreading the manure. As a consequence, there is a considerable interest in reducing the amount of P in swine manure. This can be accomplished by feeding highly digestible P-sources and by not feeding the pig more digestible P than what it requires for maximum productivity. Therefore, it is imperative that precise digestibility coefficients of P in feed ingredients be determined. By having such values, P-sources with a high digestibility can be selected, and the inclusion of P in diets for swine can be limited to only what is needed by the animal.

To measure P digestibility directly in feed phosphates for swine, a P-free basal diet is needed. Such a diet has not yet been developed. However, work at South Dakota State University has resulted in the formulation of a cornstarch-based P-free diet supplemented with gelatin. Theoretically, such a diet can be used to directly measure P digestibility coefficients in feed P for swine. However, because swine diets are usually formulated based on digestible amino acids (AA), it is necessary to measure AA digestibility coefficients in the cornstarch-based diet supplemented with gelatin before this diet can be used as a basal diet in P experiments. The objective of this experiment was to measure apparent and standardized ileal digestibility coefficients of AA in two cornstarch-based diets supplemented with gelatin or soybean meal by growing pigs.

Materials and methods

Animals and housing

Four growing barrows (BW: 44.05 ± 1.45 kg) were obtained from the South Dakota State University (SDSU) Swine Research Farm and 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 metabolism cages for the duration of the experiment in an environmentally controlled building. Room temperature was maintained at 22°C. The cages were equipped with a feeder, a nipple waterer and expanded metal slatted floors.

Once the experiment ended, pigs were moved to individual pens and housed there until they reached normal slaughter weight. At this time they were harvested at the Meat Science Lab at SDSU. The experiment was reviewed and approved by the Institutional Animal Care and Use Committee at SDSU (# 03-A012).

Diets and feeding

The gelatin sources composition is displayed in Table 3.1. The pork gelatin had a slightly higher concentration of all AA except Ile, Leu, and Thr compared to the beef gelatin. Four diets were prepared (Table 3.2 and 3.3). Diets 1 and 2 contained pork gelatin and beef gelatin, respectively. To compensate for the low concentrations of His, Ile, Met, and Trp in gelatin, both diets were supplemented with crystalline sources of these AA. Diet 3 contained SBM as the only AA-containing ingredient. Diet 4 was an N-free diet without any protein-containing ingredients. Solka floc, a synthetic source of

fiber, was included in diets 1, 2, and 4 at a level of 4% to provide fibers. Dextrose and soybean oil were included in all diets at 15% and 4%, respectively. Chromic oxide (0.4%) was included in all diets as an inert marker; vitamins and minerals were included at levels that met or exceeded the NRC requirements for growing pigs (NRC, 1998).

Feed was distributed in two daily meals at 2.5 times the maintenance requirement for energy. Water was available at all times. Every morning, orts were removed from the feeders and weighed back before new feed was supplied to the pigs.

Following the surgery, pigs were allowed a 2-wk recuperation period before the experiment was initiated. During this time, they were fed a standard corn soybean meal-based, 16 % CP grower diet.

Experimental design and digesta collection

Pigs were arranged in a 4 X 4 Latin square design with four periods and four animals representing the rows and the columns, respectively. Each experimental period lasted 7 d. The initial 5 d of each period was considered an adaptation period while the remaining 2 d were used for digesta collections in 12 h periods as described by Stein et al. (1999a). In short, a plastic bag was attached to the cannula barrel and digesta flowed into the bag and was collected. Bags were removed whenever they were filled with digesta. They were immediately frozen at –20°C to prevent bacterial degradation of the digesta proteins.

Chemical analysis

At the end of the experiment, samples were thawed, mixed within animal and diet, and a sub-sample was taken for chemical analysis. All digesta samples were freeze-dried

and finely ground prior to chemical analysis. Dry matter and CP were analyzed in digesta samples, diets, and the gelatin sources according to AOAC procedures (AOAC, 2000). Amino acids were also analyzed in these samples on a Chrom-tech HPLC AA analyzer, using ninhydrine for post-column derivatization and nor-leucine as the internal standard (AOAC, 2000). Prior to analysis, samples were flushed with N and hydrolyzed with 6 *N* HCL for 24 h at 110°C. Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight prior to hydrolysis. Tryptophan was determined after alkaline hydrolysis for 22 h at 110°C. The chromium content of diets and digesta samples were determined using spectrophotometry as described by Fenton and Fenton, (1979).

Calculations and statistical analysis

Data for feed intake were summarized and ADFI for each animal and period was calculated. Apparent ileal digestibility (AID) coefficients for AA in the two gelatin-containing diets and in soybean meal were calculated using equation [1] (Stein et al., 1999a):

$$AID = (100 - [(AAd/AAf) \times (Crf/Crd)] \times 100 \quad \textbf{[1]}$$

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 chromium content in the feed DM, and Crd is the chromium content in the ileal digesta DM. The AID of CP was calculated using the same equation.

The basal endogenous flow to the distal ileum of each AA was determined based on the flow obtained after feeding the N-free diet using equation [2] (Stein et al., 1999b):

$$EAL = [AAd \times (Crf/Crd)] \quad \textbf{[2]}$$

where EAL is the basal endogenous loss of an AA (mg/kg DMI).

By correcting the AID for the EAL of each AA, standardized ileal AA digestibility (SID) coefficients were calculated using equation [3] (Stein et al., 2001):

$$SID = [AID + (EAL/AAd)] \quad \textbf{[3]}$$

where SID is the standardized ileal digestibility coefficient (%).

Data were analyzed statistically by using the Proc Mixed procedure of SAS (Littell et al., 1996). An analysis of variance was conducted with pigs, periods, and diets as the main effects. Treatment means were separated using the LSMeans statement and the DIFF option of Proc Mixed.

Results

The pigs stayed healthy throughout the experiment and readily consumed their diets (data not shown). Data on AID and SID are represented in Tables 3.4 and 3.5, respectively. Pig and period were included in the model, but were found not to be significant. The AID for His in the beef gelatin diet was lower ($P < 0.05$) than in the other two diets; however the SID for His was not different among diets. For Ile and Trp, both the AID and the SID for SBM were lower than for the two gelatin-containing diets ($P < 0.05$). The AID for Phe was not different among diets, but the SID for Phe in SBM was lower ($P < 0.05$) than for the pork gelatin-containing diet. The AID and SID for Thr in beef gelatin were lower ($P < 0.05$) than in SBM, but the two gelatin-containing diets were not different.

The SID for Ala was not different among diets, but the AID was lower for the SBM diet than for the pork-gelatin diet ($P < 0.05$). Aspartic acid and Cys in the beef gelatin-containing diet had lower ($P < 0.05$) AID and SID than in the other two diets. The pork gelatin-containing diet also had a lower ($P < 0.05$) AID and SID for these two AA than did the soybean meal diet. The AID and SID for Glu were lowest ($P < 0.05$) in the beef gelatin diet, but there was no difference between the other two diets.

The AID for Gly in the soybean meal diet was lower than ($P < 0.05$) in the two gelatin-containing diets. There was no difference in SID of Gly between any of the diets.

The AID and SID of Tyr in beef-gelatin (42.85% and 62.25%, respectively) were lower ($P < 0.05$) than for the other two diets (81.21% and 87.33%), and (89.12% and 89.92%), respectively.

Discussion

The results of this experiment indicate that the indispensable AA in the diet based on pork-gelatin are as digestible as the AA in SBM. The same is true for the diet based on beef-gelatin with the exception of His and Thr.

In agreement with the work by Boomgaardt and Baker (1972, 1973) it was shown that gelatin, regardless of source, is devoid of Trp. In addition, gelatin contains only small amounts of Ile and the sulfur-containing AA. Therefore, the AID and SID that was measured for Trp in the gelatin-containing diets are actually the AID and SID for crystalline Trp. That explains why the SID for Trp in those diets is close to 100% and also why the gelatin-containing diets had SID for Trp that were higher than in SBM. For

Ile and Met, the AID and SID represent the digestibility of the mixture of gelatin and crystalline sources of these AA. This explains why the digestibility of Ile in both gelatin–based diets is higher than in the SBM-based diet.

For Cys, very low AID and SID were found in both gelatin-containing diets. Therefore the Cys has to be synthesized from Met. When formulating a cornstarch-based diet supplemented with gelatin, enough Met is needed to cover the Met and Cys requirement of the animal.

The reason the AID for His was lower in the beef-gelatin diet than in the pork-gelatin diet is that beef-gelatin has a lower concentration of His. Because of this, the endogenous His contributes more to the total output of His in the pigs fed the beef-gelatin-containing diet as compared to the pigs fed the pork-gelatin-containing diet. Therefore, the SID that are corrected for the endogenous losses, are not different between the two sources.

In conclusion, most AA in the two gelatin sources have AID and SID that are similar to soybean meal. Therefore, both gelatin sources are well suited for inclusion in a P-free diet provided that crystalline His, Ile, Met, and Trp, are added to balance the indispensable AA. The P-free diet allows for the estimation of endogenous losses of P by pigs, which in turn may be used to estimate the true digestibility coefficients of P in feed ingredients included in swine diets.

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Table 3.1. Analyzed nutrient composition of the gelatin used in the gelatin-containing diets (as is basis)

Nutrient, %	Pork gelatin	Beef gelatin
Dry matter	89.07	89.19
Crude protein	109.21	109.19
Indispensable AA		
Arginine	8.21	7.97
Histidine	0.88	0.59
Isoleucine	1.32	1.42
Leucine	2.93	3.03
Lysine	4.30	3.74
Methionine	1.01	0.90
Phenylalanine	2.05	1.96
Threonine	1.73	1.84
Tryptophan	<0.04	<0.04
Valine	2.42	2.43
Dispensable AA		
Alanine	8.61	9.34
Aspartic acid	5.79	5.54
Cysteine	0.16	0.06
Glutamic acid	10.18	10.74
Glycine	21.30	21.27

Proline	12.74	12.42
Serine	2.69	2.73
Tyrosine	0.71	0.28

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

Item	Pork gelatin diet	Beef gelatin diet	Soybean meal diet	N-free diet
Ingredients, %				
Gelatin, pork	30	-	-	-
Gelatin, beef	-	30	-	-
Soybean meal	-	-	40.0	-
Solka floc [a]	4.0	4.0	-	4.0
Dextrose	15.0	15.0	15.0	15.0
Soybean oil	4.0	4.0	4.0	4.0
Chromic oxide	0.40	0.40	0.40	0.40
Dicalcium phosphate	2.7	2.7	1.3	2.7
Limestone	0.15	0.15	0.6	0.15
DL-Methionine	0.34	0.34	-	-
L-Tryptophan	0.14	0.14	-	-
L-Isoleucine	0.12	0.12	-	-
L-Histidine	0.05	0.05	-	-
Salt	0.4	0.4	0.4	0.4
Vitamins premix [b]	0.1	0.1	0.1	0.1
Micro mineral premix [c]	0.1	0.1	0.1	0.1
Corn starch	42.50	42.50	43.10	78.15

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

[b] Vitamin premix provided per kg of diet: 10,032 IU of vitamin A acetate; 992 IU of vitamin D_3 as d-activated animal sterol; 88 IU of vitamin E as DL-alpha tocopheryl acetate; 1.5 mg of vitamin K as menadione dimethypyrimidinol bisulfate; 0.4 mg of biotin; 60 mg of niacin; 25 mg of pantothenic acid; 10mg of riboflavin; and 0.05 mg of vitamin B_{12}.

[c] Trace mineral premix provided per kg of diet: Cu, 23 mg as copper sulfate; Fe, 110 mg as iron sulfate; I, 0.275 mg as potassium iodate; Mn, 23 mg as manganese sulfate; Se, 0.275 mg as sodium selenite; Zn, 114 mg as zinc oxide.

Table 3.3. Analyzed nutrient composition of diets (as is basis) [a]

Nutrient	Pork gelatin diet	Beef gelatin diet	Soybean meal diet	N-free diet
Energy, Mcal ME/Kg	3,217	3,217	3,672	3,779
Dry matter,%	91.31	91.77	92.22	92.98
Crude Protein, %	35.17	32.55	20.29	0.26
Calcium, %	0.62	0.62	0.62	0.62
Phosphorus, %	0.50	0.50	0.50	0.50
Indispensable AA				
Arginine, %	2.54	2.49	1.36	0.02
Histidine, %	0.41	0.31	0.50	-
Isoleucine, %	0.97	0.99	0.89	0.01
Leucine, %	0.95	0.98	1.49	0.02
Lysine, %	1.21	1.17	1.22	0.01
Methionine, %	1.17	1.07	0.34	0.01
Phenylalanine, %	0.64	0.64	0.98	0.01
Threonine, %	1.22	1.09	1.13	0.01
Tryptophan, %	0.17	0.18	0.31	-
Valine, %	0.77	0.76	0.94	0.01
Dispensable AA				
Alanine, %	2.87	3.16	0.85	0.02

Aspartic acid, %	1.98	1.91	2.28	0.03
Cysteine, %	0.02	0.01	0.23	-
Glutamic acid, %	3.30	3.28	3.58	0.04
Glycine, %	8.06	8.07	0.83	0.01
Proline, %	4.09	4.08	0.94	-
Serine , %	0.49	0.52	0.68	0.01
Tyrosine, %	0.23	0.09	0.70	0.01

[a] Values for ME, Ca, and P were calculated, while all other values were analyzed.

Table 3.4. Apparent ileal digestibility coefficients (AID) of CP and AA (%) for experimental diets in growing pigs [a, b]

Diets	Pork gelatin diet	Beef gelatin diet	Soybean meal diet	SEM
Crude protein	82.68	81.12	84.22	1.69
Indispensable AA				
Arginine	94.15	94.02	93.04	1.17
Histidine	89.82 [x]	86.38 [y]	89.58 [x]	0.98
Isoleucine	93.17 [x]	93.03 [x]	86.52 [y]	0.84
Leucine	85.68	84.07	86.53	1.47
Lysine	89.25	90.00	88.50	1.18
Methionine	91.43	90.36	89.84	0.97
Phenylalanine	90.68	89.58	87.55	1.02
Threonine	79.09 [xy]	76.98 [y]	84.41 [x]	1.88
Tryptophan	91.51 [x]	93.23 [x]	86.61 [y]	1.13
Valine	86.36	85.99	85.28	1.44
Mean, indispensable AA	84.51 [xy]	82.79 [y]	87.69 [x]	1.53
Dispensable AA				
Alanine	86.99 [x]	85.70 [xy]	82.03 [y]	1.41
Aspartic acid	71.31 [y]	54.93 [z]	84.52 [x]	2.77
Cysteine	22.28 [x]	-161.81 [y]	81.10 [x]	18.73
Glutamic acid	81.75 [xy]	78.46 [y]	87.60 [x]	1.89

Glycine	81.64 [x]	80.67 [x]	73.19 [y]	2.10
Proline	80.42	78.20	75.12	4.32
Serine	78.76	79.15	81.44	2.07
Tyrosine	81.21 [x]	42.85 [y]	87.33 [x]	3.54
Mean, dispensable AA	83.41	80.82	83.58	1.57
Mean, total AA	83.77	81.44	85.53	1.52

[a] AID = (100-[CP or AA in digesta/ CP or AA in feed) x (Cr in feed/ Cr in digesta)]) x 100%.

[b] Data represent means of four observations.

[x, y, z] Means within a row lacking a common superscript differ ($P < 0.05$).

Table 3.5. Standardized ileal digestibility coefficients (SID) of CP and AA (%) for experimental diets in growing pigs [a, b]

Diets	Pork gelatin diet	Beef gelatin diet	Soybean meal diet	SEM
Crude protein	84.94	83.57	88.16	1.78
Indispensable AA				
Arginine	95.11	95.00	94.83	1.21
Histidine	92.07	89.29	91.42	0.94
Isoleucine	95.55 [x]	95.38 [x]	89.16 [y]	0.93
Leucine	89.22	87.48	88.80	1.54
Lysine	91.12	91.94	90.36	1.33
Methionine	91.96	90.94	91.71	1.02
Phenylalanine	93.78 [x]	91.94 [xy]	90.36 [y]	1.14
Threonine	81.97 [xy]	80.20 [y]	87.55 [x]	1.81
Tryptophan	97.20 [x]	98.55 [x]	89.66 [y]	1.02
Valine	89.82	89.52	88.15	1.55
Mean, indispensable AA	87.80	86.22	91.34	1.75
Dispensable AA				
Alanine	88.16	86.78	86.03	1.52
Aspartic acid	73.94 [y]	57.67 [z]	86.82 [x]	2.79
Cysteine	55.86 [xy]	-3.19 [y]	84.54 [x]	23.08
Glutamic acid	83.49 [xy]	80.23 [y]	89.23 [x]	1.94

Glycine	82.68	81.70	83.38	1.96
Proline	84.36	82.17	92.39	3.98
Serine	84.50	84.63	85.63	2.01
Tyrosine	89.13 [x]	62.25 [y]	89.92 [x]	4.03
Mean, dispensable AA	84.94	82.35	86.79	1.60
Mean, Total AA	85.87 [xy]	83.56 [y]	88.96 [x]	1.60

[a] SID = apparent ileal digestibility of the diet + (endogenous losses/intake) x 100%. Endogenous losses (g/kg DMI) of CP and AA were calculated as the following quantities; CP, 8.67; Arg, 0.27; His, 0.10; Ile, 0.25; Leu, 0.37; Lys, 0.25; Met, 0.07; Phe, 0.22; Thr, 0.38; Trp, 0.10; Val, 0.29; Ala, 0.37; Asp, 0.57; Cys, 0.09; Glu, 0.63; Gly, 0.91; Pro, 1.77; Ser, 0.31; Tyr, 0.20.

[b] Data represent means of four observations.

[x, y, z] Means within a row lacking a common superscript differ ($P < 0.05$).

CHAPTER 4

Phosphorus digestibility in inorganic phosphorus sources by growing pigs

ABSTRACT: An experiment was conducted to measure apparent (ATTD) and true (TTTD) total tract digestibility coefficients in five feed phosphates by growing pigs. The five feed phosphates were dicalcium phosphate (DCP), monocalcium phosphate with 70% purity (MCP70), monocalcium phosphate with 85% purity (MCP85), monocalcium phosphate with 100% purity (MCP100), and monosodium phosphate (MSP). A gelatin cornstarch-based P-free basal diet was formulated. Five additional diets were formulated by adding 0.2% total P from each of the five inorganic P-sources to the basal diet. A seventh diet was formulated by adding 0.16% P from MCP85 to the basal diet. The seven diets were fed to seven growing pigs (initial BW: 27.4 ± 2.36 kg) that were arranged in a 7 X 7 Latin square design. Each feeding period lasted 12 d. Urine and fecal samples were collected during six d of each period following the established procedures. At the completion of the experiment, samples of feed, urine, and feces were analyzed for their P concentration and ATTD for P in each feed phosphate was calculated. The ATTD of P in MSP was higher ($P < 0.05$) than for DCP, MCP70, and MCP85 (91.9 vs. 81.5, 82.6, and 81.7%, respectively), but the ATTD for MCP100 (88%) was not different from any of the other P-sources. The endogenous loss of P was measured in pigs fed the P-free basal diet and was measured at 0.139 g per kg DMI. By correcting the ATTD for the endogenous loss, the TTTD were calculated. For MSP, the TTTD was 98.2%. This value was higher ($P < 0.05$) than the TTTD for DCP, MCP70, and MCP 85 (88.2, 89.5, and 88.4%,

respectively). For MCP100, a TTTD of 94.9% was calculated – this number was not different from the TTTD for any of the other P-sources. The ATTD and the TTTD for MCP85 were identical regardless of measured in the diet containing 0.16 or 0.20% total P. Results from the current experiment suggest that the digestibility of P in DCP and various MCP sources is similar; however, they all have digestibility coefficients that are lower than current NRC estimates of 95 to 100% relative bioavailability. On the other hand, the TTTD of P in MSP is close to 100%.

Key Words: Apparent digestibility, Endogenous losses, Pigs, Phosphorus, True digestibility

Introduction

To satisfy the growing pig's requirement for phosphorus (P), it is usually necessary to include a source of inorganic P in the diets fed to growing pigs. Inorganic P usually has a higher digestibility coefficient for pigs than phytate bound P found in vegetable feed ingredients. Therefore, the inorganic P can compensate for the low digestibility of P in vegetable feed sources. Monocalcium phosphate (MCP) and dicalcium phosphate (DCP) are the main sources of inorganic phosphate used in the US feed industry. It has been shown that MCP is more digestible to pigs than is DCP (Huyghebaert et al., 1980; Jongbloed, 1987). Because sources designated as MCP and DCP in reality are mixtures of MCP and DCP, differences within a source may exist. In general, it is expected that the higher the content of MCP is, in a particular P-source, the

higher the digestibility of P in that source is. Because the digestibility is expected to increase with increasing MCP-concentration, there may be differences in the digestibility between MCP sources.

To investigate the influence of the various sources of feed P on P-digestibility, there is a need to measure differences in digestibility coefficients between sources of inorganic feed P. While usually not included in diets for livestock, MSP has often served as a standard in studies aimed at measuring P-availability; thus, MSP is also included in this experiment.

The objective of the current experiment was to compare apparent and true total tract digestibility coefficients in different inorganic P-sources by growing pigs.

Materials and methods

Experimental design

The apparent and true digestibility of five inorganic P-sources was measured in this experiment. The five P sources were dicalcium phosphate (DCP), monocalcium phosphate with 70% purity (MCP70), monocalcium phosphate with 85% purity (MCP85), monocalcium phosphate with 100% purity (MCP100), and monosodium phosphate (MSP). Dicalcium phosphate, MCP70, and MCP85 all contain varying amounts of MCP and DCP. Dicalcium phosphate contains approximately 40% MCP and about 45% DCP. Monocalcium phosphate with 70% purity contains approximately 70% MCP and 15% DCP. Monocalcium phosphate with 85% purity has 85% MCP and no DCP. Each of these P-sources was included in one of the experimental diets (Tables 4.1

and 4.2). To further investigate the difference between MCP70 and MCP85, a diet containing the same amount of MCP from MCP85 as was included in the MCP70 diet was also included. In addition, a P-free diet was included in the experiment to measure endogenous losses of P. Therefore a total of seven diets were used in this experiment.

A 7 X 7 Latin square design was used with seven periods and seven animals comprising the rows and the columns, respectively. Each pig was fed one of the diets during each period. At the conclusion of the experiment, all seven pigs had been fed each of the seven diets during one period.

Animals and housing

Seven growing barrows (initial and final body weight: 27.4 ± 2.4 kg and 78.8 ± 9.3 kg, respectively) were used in the experiment. The barrows originated from the mating of Hampshire boars to Landrace/Yorkshire sows and were obtained from the South Dakota State University (SDSU) Swine Research Farm. Pigs were individually housed in metabolism cages and randomly assigned to their treatment diets. The cages were equipped with a feeder and a nipple waterer. They had expanded metal slatted floors, a screen based floor for collection of fecal matter, and a tray for urine collection. Room temperature was maintained at 22˚C.

At the conclusion of the experiment, pigs were moved to individual pens and remained there until they reached normal slaughter weights, at which time they were harvested at the Meat Science Laboratory at SDSU. The experiment was reviewed and approved by the Institutional Animal Care and Use Committee at SDSU (# 02 – A055).

Diets and feeding

Seven experimental diets were prepared (Table 4.1 and 4.2). The basal diet (diet 1) was a cornstarch-based gelatin-supplemented P-free diet. Diets 2-6 were identical to the basal diet with the exception that each diet contained 0.2% inorganic P. The inorganic P was supplied from DCP (diet 2), MCP70 (diet 3), MCP85 (diet 4), MCP100 (diet 5), or MSP (diet 6). In diet 7, 0.16% inorganic P was included from MCP85 to make the amount of MCP equal to that in diet 3. Inorganic calcium in the form of limestone was added to all diets to bring the total to 0.3%. Other minerals, amino acids, and vitamins were included at the same concentrations in all diets at levels that met or exceeded current recommendations (NRC, 1998).

Pigs were fed at 2.5 times their maintenance requirement of metabolizable energy (106 kcal ME/kg $^{0.75}$; NRC, 1998). The daily rations were divided into two equal meals and fed to the pigs at 8:00am and 6:00pm. Water was available at all times.

Data recording and sample collection

Pig weights were recorded at the end of each period and the amount of feed supplied each d was recorded as well. Average daily weight gain and average daily feed disappearance was calculated for each diet and period at the end of the experiment.

Each feeding period lasted 12 d. The initial five d of each period was considered an adaptation period to the diet. On d 6 to 11, urine was collected in the morning and afternoon via the trays underneath the crates. All the urine collected on one d was weighed and mixed. A 20% sample was taken and frozen at -20˚C. In the morning meal on d 6 and 11, 1g chromic oxide was mixed into the meal and used as a fecal marker.

When the marker showed after d 6, total fecal collections started. When the marker showed again after d 11, fecal collections ceased. All feces collected were frozen at -20˚C. After the completion of one experimental period, the animals were deprived of feed overnight and the following morning a new experimental diet was initiated.

Chemical analysis

At the end of the experiment, urine samples and fecal samples were thawed, mixed within animal and diet, and a sub-sample was taken for chemical analysis. A sample of each diet and of each of the P-sources was taken as well. Fecal samples were dried using a forced-air drying oven set at 60˚C. All samples were finely ground prior to chemical analysis. Samples were analyzed for their moisture content (AOAC, 2000). The P-contents of all samples were analyzed using UV spectrophotometry (AOAC, 2000).

Calculations and statistical analysis

Data for feed intake were summarized and ADFI for each animal and period was calculated as was ADG.

Apparent total tract digestibility coefficients (ATTD) of P in each of the six P-containing diets (diet 2 – 7) were calculated using equation [1] (Adeola, 2001):

$$ATTD = [(Pi-Pf)/Pi] \times 100\% \quad \textbf{[1]}$$

where ATTD is the apparent total tract digestibility coefficient (%), Pi is the total P-intake from d 6 to d 11 of each experimental period (g), and Pf is the total fecal output of P during that same period (g).

Phosphorus retention (Pr) for each pig and period was calculated using equation [2] (Adeola, 2001):

$$Pr = [(Pi-(Pf+Pu))/Pi] \times 100\% \quad \textbf{[2]}$$

where Pr is the P-retention (%), and Pu is the urinary output of P from day 6 to day 11 (g).

The endogenous losses of P were calculated from diet 1 using equation [3] (Stein et al., 2001):

$$EPL = (Pf/Fi) \quad \textbf{[3]}$$

where EPL is the endogenous loss of P (mg/kg DMI), and Fi is total feed intake (kg DM).

To calculate true total tract P-digestibility coefficients (TTTD), values for ATTD and the endogenous losses were added according to equation [4] (Stein et al., 2001):

$$TTTD = ATTD + [(EPL/Pi) \times 100\%] \quad \textbf{[4]}$$

where TTTD represents true total tract digestibility coefficients (%) of P, ATTD is calculated using equation [1], and EPL is calculated according to equation [3].

Data were analyzed statistically using the Proc GLM procedures of SAS (SAS Institute, Cary, NC). The model included diet, pig, and period as the main effects. An analysis of variance was conducted to detect differences in ATTD, Pr, and TTTD between the P-containing diets. Treatment means were separated using the LSMeans statement and the Pdiff option in SAS. Homogeneity of data was confirmed using the UNIVARIATE procedure of SAS. An alpha level of 0.05 was used to assess significance between treatment means. Linear and quadratic effects for diets 2, 3, 4, and 5 were analyzed using Proc Glm.

Results

The pigs stayed healthy throughout the experiment and readily consumed their diet. Data on P-digestibility and retention are presented in Table 4.3. Pig and period were included in the model, but were not significant. There were no differences in ADFI among diets. Likewise, there were no differences in the intake of P among diets, although pigs fed the low MCP85 diet as expected had the numerically lowest P intake.

The fecal P-output was higher ($P < 0.05$) from the pigs fed the DCP diet, the MCP70 diet, and the MCP85 diet compared with the pigs fed the MSP diet. The fecal P-output from pigs fed the MCP100 diet was not different from pigs fed any of the other diets. The absorption of P did not differ among the DCP, MCP70, MCP85, MCP100, and MSP diets. However, the absorption of P for pigs fed the MSP diet was higher ($P < 0.05$) than for pigs fed the low MCP85 diet.

The ATTD of P for MSP was higher ($P < 0.05$) than for DCP, MCP70, and MCP85, but not higher than for MCP100. There was a trend ($P < 0.10$) for pigs fed MCP100 to have a higher ATTD than pigs fed DCP, MCP70, and MCP85. The ATTD for MCP100 and MSP were also higher ($P < 0.05$) than for the low MCP85 diet. A strong linear trend ($P = 0.055$) between the four MCP-containing diets and ATTD was observed.

The pigs fed the MSP diet had a higher ($P < 0.05$) urinary P-output compared with pigs fed using other diets. Also, as the amount of MCP in the MCP-containing diets increased, the urinary P-output increased linearly ($P < 0.05$).

When measured as a percentage of P-intake, pigs fed DCP, MCP70, and MCP85 had a higher ($P < 0.05$) P-retention than pigs fed MSP. There was also a trend ($P < 0.10$) for pigs fed MCP100 to have higher P-retention than pigs fed MSP. However, when measured in grams there were no differences among diets in the amount of P that was retained.

The endogenous loss of P calculated from the pigs fed the P-free diet averaged 0.139 g/kg DMI. By multiplying this amount with the feed intake of each pig, total endogenous losses were calculated for each diet. These losses did not differ among treatments.

By subtracting the endogenous losses from the total fecal P-output, the undigested dietary P in the feces was calculated. Undigested feed P in g was higher ($P < 0.05$) in pigs fed DCP, MCP70, and MCP85 than in pigs fed MSP.

True total tract digestibility coefficients of P in MSP were higher ($P < 0.05$) than in DCP, MCP70, and MCP85. The TTTD for the MCP100 diet was not different from any of the other diets. Likewise, the TTTD for DCP, MCP70 and MCP85 were similar.

Discussion

By feeding the P-free diet to pigs, the endogenous losses of P were estimated directly. Previous estimates for endogenous losses have been 90-630 mg/kg DMI (Ajakaiye et al., 2003); 570-840 mg/kg DMI (Shen et al., 2002); 140-320 mg/kg DMI (Fan et al., 2001); 70 mg/kg DMI (Pettey et al., 2003); and 70-800 mg/kg DMI (Jongbloed, 1987). All previous data on endogenous losses of P were estimated by

feeding several levels of P and then regressing back to zero P-intake. In the present experiment the endogenous losses were measured directly in the pigs. To our knowledge, this is the first time such a technique has been reported. Nevertheless, data from the current experiment (139 mg/kg DMI) agree well with Fan et al. (2001) and Ajakaiye et al. (2003), but they are considerably lower than the values provided by Shen et al. (2002) and higher than those reported by Pettey et al. (2003). However, Jongbloed pointed out that the amount of endogenous P lost in the feces is dependent on the dietary level of P. If the P-intake is considerably below the P-requirement, low values for endogenous P would be expected (Jongbloed, 1987). Because a P-free diet was used in this experiment, the values obtained may be considered the minimal or basal endogenous losses of P from growing pigs.

There was no difference in the digestibility between the MCP-containing sources. The ATTD for DCP found in this study was higher than previous estimates (Jongbloed et al., 1991). However, differences exist among DCP sources which may be the reason for this discrepancy (Jongbloed et al., 1991). Most sources of MCP are a mixture of MCP and DCP in varying amounts. It has previously been shown that MCP has a higher digestibility than DCP (Jongbloed, 1987). Therefore, a higher content of MCP is expected to increase the digestibility. However, if the difference in digestibility between MCP and DCP is small, as was the case in this experiment, then the differences between MCP sources containing different amounts of DCP will also be small. This explains why no difference between MCP70 and MCP85 was found in this experiment.

Cromwell (1992) found that the difference in relative bioavailability between DCP and MCP is 5%, with DCP having a higher relative bioavailability. In contrast, Jongbloed (1991) found digestibility values of 69% in DCP and 75-84% in MCP. In the same experiment there were differences between MCP sources as well. Rodehutscord et al. (1994) found digestibility coefficients of 91% for MCP and 87% for DCP. The values for the digestibility of DCP and MCP found in the current experiment are within the range reported by Jongbloed (1987), and Rodehutscord (1994).

The estimation of endogenous P allowed for the calculation of true P-digestibility coefficients in the inorganic P-sources. To our knowledge, such values have never been presented before. It is believed that digestibility coefficients based on TTTD are more additive in a mixed ration than are values based on ATTD (Fan et al., 2001). Therefore, TTTD for P in various feed ingredients are needed.

There was no difference between the P-sources in P-retention. This is probably because the animals fed the DCP and MCP diets were fed close to their requirement for P. When they were fed the MSP diet, they absorbed more, but the extra P was not needed and, therefore, excreted in the urine. This observation suggests that the digestibility coefficient of P is not dependent on the P-level in the diet and animals do not need to be fed below their requirement if digestibility coefficients for P are determined. Monosodium phosphate had the highest TTTD and was almost completely digested. Therefore, very low quantities of dietary P ended up in the feces of pigs fed the diet containing MSP. The values for TTTD that were calculated for the other diets were

lower than for MSP. Therefore, more dietary P is excreted in the feces if these diets are used.

However, all the values for both ATTD and TTTD obtained in this experiment are relatively high and support the hypothesis that P in inorganic P-sources has a high digestibility. In contrast, the TTTD in corn is only 54 to 60% (Shen et al., 2002), and soybean meal it is 49 to 59% (Fan et al., 2001, Ajakaiye et al., 2003).

Implications

With current regulations for P in manure, a better way to measure digestibility of P is needed to reduce the excretion of P from pigs. The formulation of a P-free diet allows for the calculation of endogenous losses, and therefore also of TTTD. With these calculations, excretion can accurately be determined, allowing for the formulation of a diet closer to the pigs' requirement. This gives the producers a way of reducing excretion of P into the environment.

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Table 4.1. Ingredient composition (%) of experimental diets (as is basis)

Ingredient (%):	Diet: 1	2	3	4	5	6	7
	P-Source: P-free	DCP[a]	MCP70[a]	MCP85[a]	MCP100[a]	MSP[a]	MCP85[a]
DCP[a]	-	1.1	-	-	-	-	-
MCP70[a]	-	-	1.1	-	-	-	-
MCP85[a]	-	-	-	0.97	-	-	0.8
MCP100[a]	-	-	-	0	0.85	-	-
MSP[a]	-	-	-	-	-	0.8	-
Potassium carbonate	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Magnesium oxide	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Solka floc[b]	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Gelatin	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Cornstarch	40.49	38.99	39.89	39.94	40.02	40.09	40.04
Sucrose	19.0	20.0	19.0	19.0	19.0	19.0	19.0
Soybean oil	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Limestone	0.8	0.2	0.3	0.38	0.42	0.8	0.45
DL-Methionine	0.34	0.34	0.34	0.34	0.34	0.34	0.34
L-Tryptophan	0.14	0.14	0.14	0.14	0.14	0.14	0.14
L-Histidine	0.05	0.05	0.05	0.05	0.05	0.05	0.05
L-Isoleucine	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Salt	0.4	0.4	0.4	0.4	0.4	-	0.4

Vitamin premix[c]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Mineral premix[d]	0.1	0.1	0.1	0.1	0.1	0.1	0.1

[a] DCP= dicalcium phosphate; MCP70= monocalcium phosphate with 70% purity; MCP85= monocalcium phosphate with 85% purity; MCP100= monocalcium phosphate with 100% purity; MSP= monosodium phosphate.

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

[c] Vitamin premix provided per kg of diet: 10,032 IU of vitamin A acetate; 992 IU of vitamin D_3 as d-activated animal sterol; 88 IU of vitamin E as alphatocopherol acetate; 1.5 mg of vitamin K as menadione dimethypyrimidinol bisulfate; 0.4 mg of biotin; 60mg of niacin; 25 mg of pantothenic acid; 10 mg of riboflavin; and 0.05 mg of vitamin B_{12}.

[d] Trace mineral premix provided per kg of diet: Cu, 23 mg as copper sulfate; Fe, 110 mg as iron sulfate; I, 0.275 mg as potassium iodate; Mn, 23 mg as manganese sulfate; Se, 0.275 mg as sodium selenite; Zn, 114 mg as zinc oxide.

Table 4.2. Nutrient composition of experimental diets (as is basis)[a]

Nutrient: Diet:	1	2	3	4	5	6	7
P-source:	P-Free	DCP[b]	MCP70[b]	MCP85[b]	MCP100[b]	MSP[b]	MCP85[b]
Energy, Mcal ME/kg	3282	3259	3258	3260	3263	3266	3264
Crude protein, %	27	27	27	27	27	27	27
Calcium, %	0.30	0.31	0.30	0.31	0.30	0.30	0.31
Phosphorus,%	0.00	0.20	0.20	0.20	0.20	0.20	0.17
Lysine, %	1.23	1.23	1.23	1.23	1.23	1.23	1.23
Methionine, %	0.57	0.57	0.57	0.57	0.57	0.57	0.57
Threonine, %	0.66	0.66	0.66	0.66	0.66	0.66	0.66
Tryptophan, %	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Histidine, %	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Leucine, %	0.93	0.93	0.93	0.93	0.93	0.93	0.93
Isoleucine, %	0.51	0.51	0.51	0.51	0.51	0.51	0.51
Valine, %	0.72	0.72	0.72	0.72	0.72	0.72	0.72
Phenylalanine, %	0.63	0.63	0.63	0.63	0.63	0.63	0.63
Arginine, %	2.49	2.49	2.49	2.49	2.49	2.49	2.49

[a] Values for P are analyzed values, while all other values are calculated.

[b] DCP= dicalcium phosphate; MCP70= monocalcium phosphate with 70% purity; MCP85= monocalcium phosphate with 85% purity; MCP100= monocalcium phosphate with 100% purity; MSP= monosodium phosphate

Table 4.3. Phosphorus digestibility of inorganic phosphorus sources by growing pigs[ab]

Response:	Diet:	2	3	4	5	6	7	SEM	*P-value*	
	P-source:	DCP[c]	MCP70[c]	MCP85[c]	MCP100[c]	MSP[c]	MCP85[c]		Linear effect[d]	Quadratic effect[d]
Feed intake, g DM		7646	7649	7810	7813	7836	7751	839	0.92	0.86
P intake, g		15.36	15.41	15.59	15.59	17.23	13.13	1.67	0.96	0.87
Fecal P, g output		2.75^{x}	2.60^{x}	2.53^{x}	1.95^{xy}	1.32^{y}	2.3^{x}	0.32	0.10	0.51
Absorption, g		12.54^{xy}	12.81^{xy}	12.37^{xy}	13.64^{xy}	15.91^{y}	10.80^{x}	1.56	0.70	0.76
ATTD[e], %		81.49^{xy}	82.55^{xy}	81.68^{xy}	87.96^{yz}	91.88^{z}	81.11^{x}	2.18	0.05	0.21
Urine P, g output		0.18^{x}	0.19^{x}	0.54^{x}	0.53^{x}	4.12^{y}	0.20^{x}	0.61	0.04	0.96
P retention, g		12.36	12.62	11.83	13.11	11.79	10.60	1.57	0.83	0.74
P retention, %		80.22^{x}	81.35^{x}	78.17^{xy}	84.77^{x}	68.49^{y}	79.70^{x}	3.66	0.29	0.22
Endogenous P loss, g		1.06	1.06	1.04	1.09	1.09	1.08	0.12	0.92	0.86
Undigested dietary P loss		1.69^{x}	1.53^{x}	1.49^{x}	0.86^{xy}	0.23^{y}	1.25^{x}	0.30	0.07	0.44
TTTD[e], %		88.41^{x}	89.45^{x}	88.64^{x}	94.93^{xy}	98.20^{y}	89.32^{x}	2.24	0.07	0.25

[a] Data represent means of seven observations.

[b] Data represents total balance over 5 days.

[c] DCP= dicalcium phosphate; MCP70= monocalcium phosphate with 70% purity; MCP85= monocalcium phosphate with 85% purity; MCP100= monocalcium phosphate with 100% purity; MSP= monosodium phosphate.

[d] Linear and quadratic effects of the inclusion of P from MCP in diets 2-5.

[e] ATTD= apparent total tract digestibility coefficient for phosphorus; TTTD= true total tract digestibility coefficient for phosphorus.

[x, y, z] Means within a row lacking a common superscript are different ($P < 0.05$)

CHAPTER 5

Relative phosphorus availability in inorganic phosphorus sources by growing pigs

ABSTRACT: An experiment was conducted to determine the relative bioavailability of five inorganic phosphorus (P) sources by growing pig diets. The five P-sources were dicalcium phosphate (DCP), monocalcium phosphate with 70% purity (MCP70), monocalcium phosphate with 85% purity (MCP85), monocalcium phosphate with 100% purity (MCP100), and monosodium phosphate (MSP). The experiment was set up as a 5x2 factorial with five P-sources and two added P-levels (0.07 and 0.14% P) for each P-source. A basal diet (0.10%P) was included. Forty four growing pigs (initial BW 16.8 ± 4.3 kg) were randomly allotted to the 11 experimental diets and housed in individual grower pens. Feed was provided on an ad libitum basis throughout the 28 d experimental period. At the conclusion of the experiment, all pigs were euthanized and a blood sample and six bones (i. e., tibia, fibula, and third and fourth metacarpals on both front feet) were harvested. Blood samples were analyzed for Ca and P concentrations. Bone breaking strength, bone ash, and the Ca and P concentrations were determined in the metacarpals. Bone density measurements were obtained using two machines, Dual-energy X-ray Absorptiometry (DXA) and Peripheral Quantitative Computed Tomography (pQCT). Correlation coefficients and prediction equations from DXA and pQCT to breaking strength were obtained. The relative bioavailability of P in each of the five sources was determined using slope ratio methodologies based on breaking strength. The DCP,

MCP70, MCP85, and MCP100 sources had P-availabilities of 49.5, 68.9, 66.3, and 84.9%, respectively, relative to MSP. The slope of the regression line for diets containing MSP was steeper ($P < 0.05$) than the slopes for pigs fed diets containing DCP, MCP70, and MCP85, but not different from that of pigs fed diets supplemented with MCP100. The slope for pigs fed diets containing MCP100 was also steeper than the slope for pigs fed diets containing DCP ($P < 0.05$), but not different from pigs fed diets containing other sources of P. It was shown that bone mineral content and bone mineral density most precisely predicted breaking strength. In conclusion, P in MSP is more available than in DCP and MCP, but there were no significant differences within MCP sources.

Key words: Bone ash, Bone breaking strength, Bone density, Phosphorus, Pigs, Relative bioavailability

Introduction

To satisfy the growing pigs' requirement for P, it is usually necessary to include a source of inorganic P in the diets fed to growing pigs. Inorganic P usually has a higher digestibility for pigs than has organic P from vegetable feed ingredients. Therefore, the inorganic P can compensate for the low digestibility of P in vegetable feed sources. Monocalcium phosphate (MCP) and dicalcium phosphate (DCP) are the main sources of inorganic P supplements used in the US feed industry. It has been reported that MCP is more digestible in pigs than DCP (Huyghebaert et al., 1980). However, because sources

designated as MCP or DCP in reality are mixtures of MCP and DCP, differences within a source may exist. In praxis, inorganic P-sources designated as MCP may contain between 70% and 85% MCP. In general, it is expected that the higher the content of MCP the higher the availability of P in that source is. Likewise, if water is attached to the P molecule, there is a higher availability than if no water is attached (Grimbergen et al., 1985). However, in a recent experiment it was found that no difference in the digestibility of P exists between DCP and different MCP-sources (current thesis, chapter 4). The current experiment was conducted to validate these results. The objective was to compare the relative availabilities of P in five inorganic P sources fed to growing pigs, and to compare bone-breaking strength to bone density parameters.

Materials and methods

Animals, experimental design, and diets

The animal part of the experiment lasted 28 d and was conducted as a 5 by 2 factorial design with five different P-sources and two different inclusion levels. The P-sources used were dicalcium phosphate (DCP), monocalcium phosphate with 70% purity (MCP70), monocalcium phosphate with 85% purity (MCP85), monocalcium phosphate with 100% purity (MCP100), and monosodium phosphate (MSP). A low-P basal diet was also included. Therefore, a total of 11 different diets were used. There were four replications of each treatment diet, thus, a total of 44 pigs were used in this assay. All pigs (initial BW: 16.8 ± 4.3 kg) were penned in an individual grower pen equipped with a slatted floor, a feeder, and a nipple waterer. Pigs had ad libitum access to water and feed.

At the start of the experiment, pigs were randomly assigned to one of the 11 dietary treatments.

A low-P basal diet (diet 1) was formulated using corn, corn starch, sucrose, potato protein, and casein as the main feed ingredients (Tables 5.1 and 5.2).

The basal diet was formulated to contain 0.10% P. For each source of inorganic P, two diets were formulated by adding 0.07 or 0.14% inorganic P to the basal diet. Thus, in diets 2 and 3, DCP was added to the basal diets at 0.38 and 0.76%, respectively, to provide 0.07 and 0.14% extra P. This brought the calculated total P-content of these diets to 0.17 and 0.24%, respectively. Likewise, in diets 4 and 5, MCP70 was added to the basal diet to bring the calculated P contents of these diets to 0.17 and 0.24%, respectively. Diets 6 and 7 included MCP85 and diets 8 and 9 included MCP100 to reach the same levels of P. Diets 10 and 11 were identical to the basal diet with the exception that MSP was added at 0.27 and 0.54% of the diet, respectively to provide a total of 0.17 and 0.24% P in the diets. In all diets, the P-source was included at the expense of corn starch. Limestone was used as an inorganic Ca source and included at varying concentrations to bring the Ca: P ratio in all diets to 1.3:1. Solka floc was included in all diets as a source of fiber, while vitamins and minerals other than Ca and P were included at levels that met or exceeded NRC recommendations (NRC, 1998). The experiment was reviewed and approved by the Institutional Animal Care and Use Committee at South Dakota State University (# 04 – E005).

Sample collections, measurements and chemical analysis

Daily feed allotments were measured, as was initial and final BW of the pigs. At the conclusion of the experiment, pigs were euthanized by electrocution and the third and fourth metacarpals on both front feet were removed. In addition, the tibia and fibula from one hind leg was recovered for bone density analysis. All bones were individually packaged in plastic bags and frozen until they were ready to be analyzed. Prior to euthanization, a blood sample was drawn from each pig and serum was harvested to determine the Ca and P concentration in the serum. All diets were analyzed for their concentrations of DM, CP, amino acids (AA), Ca, and P (AOAC, 2000).

Bone breaking strength was determined on the third and fourth metacarpals in the two forelimbs of each pig. The breaking strength was measured on an Instron instrument (Instron Corp., Canton, MA). The Instron instrument measures kilograms of force required to break the metacarpal when placed on supports 3.5 cm apart. Force was applied to the shaft of the bone by an instrument moving at 5cm/min and measured by a pressure-sensitive cell and recorded on a graph recorder.

Broken bones were then defatted via ether extract for 3 d and ashed in a muffle furnace at 600ºC for 16 h for the determination of total bone ash and bone concentrations of Ca and P.

Bone density was determined in the tibia by scanning the hind leg of each pig using Dual-energy X-ray Absorptiometry (DXA) and Peripheral Quantitative Computed Tomography (pQCT). The left forearm scan mode of the DXA scanner (Hologic QDR 4500, Hologic Inc, Waltham, MA) was used. The bone mineral content (BMC) and the

bone mineral density (BMD) were determined in the tibia in a standardized manner using the Sub-Regional Analysis Mode. The DXA scanner measures the bone in five sections. The system starts with the distal tibia and ends at the proximal tibia. The R1 area is 0 – 20% from the distal tibia, the R2 area is 20 – 40% from the distal tibia, the R3 area is 40 – 60% from the distal tibia, the R4 area is 60 – 80% from the distal tibia, and the R5 area is 80 – 100% from the distal tibia. On the pQCT densitometer (XCT 2000, Norland Medical Systems, Inc., Fort Atkinson, WI), a scout view was obtained to set a reference line at the proximal end of the tibia. Three slices were imaged at 10%, 50% and 70% of the length of the bone from this reference line (Figure 5.1). The bone length used was obtained from the DXA scan analysis. Images were acquired using 0.30 voxel; 15mm/sec scan speed and 1 block (1 rotation). The 10% slice was assumed to contain trabecular bone, and the 50% and 70% slices were assumed to contain cortical bone. Analysis of the trabecular bone used Contour Mode 2 and Peel Mode 2 and for the cortical bone, the Separation Mode 1 was used with 710 mg/cm^3 as the threshold for density and 280 mg/cm^3 as the threshold for bone strength indices. The voxel size is the dimension of one picture unit represented by an attenuation value. A total of fifty parameters were measured between the two machines.

Calculations and statistical analysis

At the conclusion of the experiment, feed consumptions for each pig were summarized to calculate ADFI. Initial BW was subtracted from final BW to calculate ADG. The average daily G: F was also calculated for each pig.

Data for pig performance, serum Ca and serum P concentration, serum Ca: P ratio, bone breaking strength, bone ash, bone Ca and bone P concentration, bone Ca: P ratio, and bone density parameters were analyzed as a complete randomized block design with a 5 by 2 factorial and an extra diet with treatments consisting of the five P sources and two levels of added P (0.07 and 0.14%). The effect of dietary P was analyzed using ANOVA in the Proc Mixed procedure in SAS (SAS Institute, Cary, NC). The main effects were P source, P level, and source by level interactions. Average values for each P-source were calculated across levels and compared using an ANOVA in Proc Mixed. Treatment metacarpal breaking strength was regressed on added P intake from each P-source using a regression analysis in Proc Mixed. The basal diet was included in all five regressions. Slopes of the resulting regression lines were determined and compared among the five P-sources to express the bioavailability of P in DCP, MCP70, MCP85, and MCP100 relative to the bioavailability of P in MSP as described by Cromwell (1992). Slope ratio analyses were analyzed using Proc Reg in SAS. Correlations between each of the bone density parameters and the breaking strength were analyzed using Proc Corr in SAS. To estimate the variation in the measurements obtained with the pQCT and DXA scan, a principal component analysis was conducted using Prin Comp in SAS. Initially, all parameters from the DXA and pQCT scan were included in the analysis. Following this initial analysis, a similar analysis was conducted with data only from the DXA scan. Finally, the data obtained from the pQCT scan were used for a similar analysis. To predict the breaking strength from the data obtained with the pQCT and DXA scan, a step-wise reduction of the model in Proc GLM was conducted and

prediction equations were obtained. In all analyses, the pig was the experimental unit and 0.05 was used as the alpha level.

Results

The pigs stayed healthy throughout the experiment and readily consumed their diets. One pig, on the low MCP70 diet, had to be removed from the experiment five d before the completion of the study. This pig was disregarded in all calculations.

The analyzed concentrations of Ca and P in the diets were close to the intended levels and the differences between low and high concentrations were as expected for all five sources of P (Table 5.2). Likewise, the concentration of CP and AA were close to expected values.

Neither source of P, nor the level of P influenced ADG, ADFI, or G: F (Table 5.3). The concentration of P in the diets influenced ($P < 0.0001$) total P-intake, serum P concentration, and serum Ca: P ratio. Phosphorus source had a significant effect ($P < 0.04$) on serum Ca concentration and serum P concentration, but not on P-intake. There was a significant ($P < 0.001$) source by level interaction for serum Ca concentration with pigs fed the high MSP diet having a lower serum Ca concentration than pigs fed the low MSP diet. Total P intake was lowest ($P < 0.05$) for pigs fed the basal diet compared to diets supplemented with P (Table 5.4). Average daily feed intake was also lower ($P < 0.05$) for pigs fed the basal diet than for pigs fed diets containing MCP70 or MCP85, but not different from that achieved on diets containing the other sources of P. Serum Ca concentration was lower ($P < 0.05$) in pigs fed the basal diet compared to pigs fed the

diets supplemented with MCP70 and MCP100, but similar to pigs fed diets containing DCP, MCP85, and MSP. Serum P concentration was higher ($P < 0.05$) in pigs fed diets containing MCP100 than in pigs fed diets supplemented with DCP and in pigs fed the basal diet, but not different from pigs fed diets containing MSP, MCP85, or MCP70. The serum P concentration for pigs fed the basal diet was also lower ($P < 0.05$) than in all diets supplemented with P except for the diets containing DCP.

The source of P influenced ($P \leq 0.05$) metacarpal bone ash content, metacarpal breaking strength, total bone Ca concentration, and total bone P concentration, but not the concentration of bone ash, amount of bone Ca, amount of bone P, or the bone Ca: P ratio (Table 5.5). The level of P in the diets influenced the concentration of bone ash, total bone Ca concentration, total bone P concentration, total bone Ca: P ratio, and the amount of ash, Ca, and P ($P < 0.05$). In addition, there was a strong tendency for an effect of P level on breaking strength ($P = 0.08$). There was a source by level interaction ($P \leq 0.04$) on the concentration of bone ash and the amount of bone ash with pigs fed the high MSP diet having lower concentration of bone ash and lower total amount of bone ash than pigs fed the low MSP diet. The metacarpal ash concentration was higher ($P \leq 0.05$) in pigs fed diets containing MSP, MCP100, MCP85, MCP70, and DCP than in pigs fed the basal diet (Table 5.6). The amount of metacarpal ash was higher ($P < 0.05$) in pigs fed diets supplemented with MSP, MCP100, MCP85, and MCP70 than in pigs fed diets containing DCP and in pigs fed the basal diet. Metacarpal bone breaking strength was higher ($P < 0.05$) in pigs fed diets containing MSP, MCP100, MCP85, MCP70, and DCP than pigs fed the basal diet; pigs fed diets supplemented with MSP also had higher breaking

strength ($P < 0.05$) than pigs fed diets containing DCP, but not higher than pigs fed the MCP-containing diets. The total bone Ca concentration was higher ($P < 0.05$) in pigs fed diets containing MCP100, MCP85, and pigs fed the basal diet compared to pigs fed diets containing DCP, but it was not different ($P > 0.05$) from pigs fed diets containing MCP70 and MSP. Total bone P concentration was higher ($P < 0.05$) in pigs fed diets containing MSP, MCP100, and MCP85 than in pigs fed all other diets. The Ca: P ratio was higher ($P < 0.05$) in pigs fed the basal diet compared to pigs fed diets supplemented with P. The total amount of bone Ca was higher ($P < 0.05$) in pigs fed diets containing MSP, MCP100, and MCP70 than in pigs fed the basal diet, but not different ($P > 0.05$) from pigs fed diets containing MCP85 and DCP. The amount of bone P was higher ($P < 0.05$) in pigs fed diets containing MSP, MCP100, MCP85, and MCP70 than in pigs fed the basal diet, but not different ($P > 0.05$) from pigs fed diets containing DCP.

The slopes of the regression lines for breaking strength are presented in Figure 5.2. The P in pigs fed diets containing DCP, MCP70, MCP85, and MCP100 had bioavailabilities of 49.5, 68.9, 66.3, and 84.9%, respectively, relative to the P in MSP. The slope of the regression line for diets containing MSP was significantly steeper ($P < 0.05$) than the slopes for pigs fed diets supplemented with DCP, MCP70, and MCP85, but not different from that of pigs fed diets supplemented with MCP100. The slope for pigs fed diets supplemented with MCP100 was also steeper than the slope for pigs fed diets containing DCP ($P < 0.05$), but not different from any of the pigs fed diets containing other sources of P.

There was no effect of P-source on any of the bone density parameters measured by the DXA scan (Table 5.7). However, P level had an effect ($P \leq 0.03$) on global BMC, global BMD, R1 BMD, R3 BMC, R4 BMC, and polar strength strain indices at the 50% slice. There was a source by level interaction ($P \leq 0.04$) for R4 BMC with pigs fed the high MSP diet having a lower BMC than pigs fed the low MSP diet. Global BMC, global BMD, and R1 BMD were higher ($P < 0.05$) in pigs fed diets containing MSP, MCP100, MCP85, MCP70, and DCP than in pigs fed the basal diet (Table 5.8). The R3 and R4 BMC was higher ($P < 0.05$) in pigs fed diets containing MSP, MCP100, MCP85 and MCP70 than in pigs fed the basal diet, but not higher ($P > 0.05$) than in pigs fed diets containing DCP. Polar strength strain indices at the 50% slice were higher ($P < 0.05$) in pigs fed diets containing MCP70 than in the basal diet, but did not differ from pigs fed diets containing other sources.

The source of P also influenced ($P \leq 0.01$) trabecular BMD (Table 5.9), but not any of the other parameters measured by the pQCT scan. The P level influenced ($P < 0.01$) trabecular BMD, cortical BMC, cortical thickness, cortical area, and total BMC at the 50% slice. There was no significant level x source interaction for any of the pQCT measurements. The trabecular area was higher ($P < 0.05$) in diets containing MSP than in diets containing any other sources (Table 5.10). Cortical BMC, cortical thickness, cortical area, and total BMC at the 50% slice were higher ($P < 0.05$) in pigs fed diets containing MSP, MCP100, MCP85, and MCP70 than in the basal diet, but not different from pigs fed diets containing DCP. Endosteal circumference at the 50% slice was higher ($P < 0.05$) in pigs fed diets containing DCP than in pigs fed diets containing MSP,

but not different from pigs fed diets containing any other sources. The cortical area at the 70% slice was higher ($P < 0.05$) in pigs fed diets containing MCP85 compared to pigs fed the basal diet.

A correlation between metacarpal bone breaking strength and certain density parameters exists (Table 5.11). Global BMC, global BMD, R1 BMD, R2 BMD, R3 BMC, R3 BMD, R4 BMC, R4 BMD, cortical BMC, cortical area, cortical thickness, total BMD, and total BMC of bone at the 50% bone slice and cortical BMC at the 70% slice are correlated with metacarpal bone breaking strength with R^2 values of 0.70 or higher. The R^2 for the correlation between the polar strength strain indices at the 50% slice and breaking strength was 0.58.

When using multiple parameters to predict bone breaking strength from data obtained with the DXA and pQCT scan, the principal component analysis showed that 94% of the variation in the model can be explained by using global BMC, endosteal circumference, R1 area, trabecular area at the 10% slice, trabecular BMD at the 10% slice, cortical area at the 70% slice, polar area moment of inertia of the area at the 50% slice, and R2 bone BMD (Table 5.12). When using only the DXA, global BMC, R2 BMD, R5 area, R1 BMD, and R5 BMC will explain 95% of the variation in the model (Table 5.13). The pQCT will explain 95% of the variation in the model when using total BMC at the 50 % slice, endosteal circumference at the 50% slice, trabecular area at the 10% slice, endosteal circumference at the 70% slice, cortical BMD at the 70% slice, and trabecular BMD at the 10% slice (Table 5.14).

When using both machines, bone breaking strength can be predicted with a R^2 of 0.85 using the following equation (Table 5.15):

y = (Periosteal circumference, 10% slice * 0.45) + (Total area, 70% slice * - 0.16) + (Cortical BMD, 70% slice * - 0.07) + (Cortical area, 70% slice * - 0.80) + (Total BMC, 50% slice * - 2.86) + (Total BMD, 50% slice * 0.40) + (Total area, 50% slice * - 5.87) + (Cortical thickness, 50% slice * 325.83) + (Endosteal circumference, 50% slice * 43.16) + (Polar moment of resistance of the cortical area, 50% slice * 0.29) + (Moment of inertia standardized weight displacement in the bone, 50% slice * 0.03) + (Polar strength strain indices, 50% slice * - 0.37) - 1050.77.

If only four parameters are used for predicting bone breaking strength the R^2 is reduced to 0.72 for the following equation:

y = (Total BMC, 50% slice * - 2.86) + (Total BMD, 50% slice * 0.40) + (Cortical thickness, 50% slice * 325.83) + (Moment of inertia standardized weight displacement in the bone, 50% slice * 0.03) - 84.01

The pQCT can predict bone breaking strength with an R^2 value of 0.85, by using the following equation (Table 15):

y = (Periosteal circumference, 10% slice * 0.45) + (Total area, 70% slice * - 0.16) + (Cortical BMD, 70% slice * - 0.07) + (Cortical area, 70% slice * - 0.80) + (Total BMC, 50% slice * - 2.86) + (Total BMD, 50% slice * 0.40) + (Total area, 50% slice * - 5.87) + (Cortical thickness, 50% slice * 325.83) + (Endosteal circumference, 50% slice * 43.16) + (Polar moment of resistance of the cortical area, 50% slice * 0.29) + (Moment of inertia

standardized weight displacement in the bone, 50% slice * 0.03) + (Polar strength strain indices, 50% slice * - 0.37) - 1050.77.

If only four parameters are used for predicting bone breaking strength, the R^2 is reduced to 0.74 for the following equation:

y = (Total BMC, 50% slice * - 2.86) + (Total BMD, 50% slice * 0.40) + (Cortical thickness, 50% slice * 325.83) + (Moment of inertia standardized weight displacement in the bone, 50% slice * 0.03) - 84.01

The DXA can predict bone breaking strength with a R^2 value of 0.70, by using the following equation (Table 15):

y = (Global BMD * 99.82) + (R4 BMD * 114.51) + (R5 area * 29.32) + (R5 BMC * - 76.90) + (R5 BMD * 392.46) - 186.56.

If only four parameters are used for predicting bone breaking strength, the R^2 is reduced to 0.66 for the following equation:

y = (R4 BMD * 174.29) + (R5 area * 37.37) + (R5 BMC * - 96.65) + (R5 BMD * 544.82) - 233.32

Discussion

The ADG was not affected by P-level or P-source. However, there was a trend (*P* = 0.13) for the ADG to increase with higher P-levels. The same trend was shown by Spencer et al. (2000) between a basal diet and a MSP standard. Cromwell et al. (1972) reported that a lower P inclusion results in slower and less efficient gains.

Serum concentrations of Ca and P increased as the concentration of MCP in the diet increased. Granner (2000) reported that plasma Ca concentrations are maintained within very narrow limits, but the current data indicate that serum Ca concentration is somewhat influenced by dietary Ca concentration. However, serum P concentrations were more influenced by the diet than were serum Ca concentrations, which were also reported by Plumlee et al. (1958) and by Peeler (1972). This observation indicates that serum P concentrations are less regulated than are serum Ca concentrations. It was also shown in the current experiment that the serum Ca:P ratio is reduced when more digestible P is added to the diet, which further indicates that P is regulated less tightly than is Ca.

The bone breaking strength values obtained in this study are similar to those reported by Coffey et al. (1994). Hall et al. (1991) reported that pigs fed a diet deficient in P have lower bone breaking strength than pigs fed a diet adequate in P. In the current experiment, bone breaking strength tended to increase ($P = 0.08$) with the level of P in the diet. The results clearly demonstrate that the P in the five sources has different availabilities with MSP having the highest availability and DCP the lowest. This response is consistent with the values reported by Coffey et al. (1994) and Hall et al. (1991). The ranking among the P-sources that was found for breaking strength is similar to the ranking based on total tract digestibility coefficients in the same five P-sources (current thesis, chapter 4). In the current experiment, a P-deficiency was created and all diets were supposed to be below the requirement for P by growing pigs. Cromwell et al. (1972) reported that increases in bone breaking strength are caused by increases in bone

thickness. If more P is absorbed and reaches the bone, then the bone will become thicker. Increases of P above the pigs' requirement do not result in increased bone breaking strength (Cromwell et al., 1972). The breaking strength for the diet with the highest inclusion of MSP was lower than expected. That may indicate that pigs fed this diet were at or slightly above their requirement for digestible P.

Bone ash concentration increased with increasing dietary P-level, but there was no effect of P-source. The quantity of ash increased with increases in P-level. This demonstrates that an increase in the availability of P will increase the size of the bone, and therefore, increases the amount of ash in the bone. The bone ash concentration is influenced by this to a lesser extent. This demonstrates that the regulation is mainly in the size of the bone, and not in the composition of the bone. This is supported by the data on bone Ca and P concentration, and the Ca:P ratios in the bone. Cromwell et al. (1972) found the correlation between bone ash concentration and bone breaking strength to be low (0.12 - 0.29). The current data support this hypothesis.

The bioavailability of P in DCP and the three sources of MCP relative to MSP indicate that P in DCP has an availability of P that is only half that of P in MSP. This is considerably lower than previous suggestions (Cromwell, 1992; Grimbergen et al., 1985; Nelson et al., 1990). Likewise, the results for the availability of P in MCP were lower than suggested by NRC (1998) and others (Grimbergen et al., 1985; Nelson et al., 1990), but they are close to values reported by Jongbloed et al. (1991). The reason why this study gave lower values for relative P-availability in DCP and MCP may be that the MSP source used in this experiment has a higher availability than what was used in previous

studies. This hypothesis is supported by the fact that bone ash concentration and bone Ca and P did not increase between the two levels of MSP indicating that the pigs' requirement may have been met at the lowest inclusion level. The MSP used in this experiment was analytical grade, but the quality of MSP in other studies was not reported. There were no differences in any of the parameters measured between MCP70, MCP85, and MCP100, although the data for MCP100 in most cases were numerically higher than the data from the other two sources. These results indicate that the concentrations of MCP in a feed phosphate designated as MCP do not significantly influence the availability of P. However, the availability of P in MCP100 was higher than in DCP. These results agree with data obtained for the total tract digestibility of P from the same five sources of P (current thesis, chapter 4). Combined, the data for relative P-availability in DCP and the three MCP sources suggest that the values for DCP and MCP given by NRC may be overestimated.

The bone density parameters were less effective in predicting differences in P-availability than was breaking strength. With the exception of trabecular BMD at the 10% slice, no differences between sources were detected. However, most of the parameters that were measured were able to distinguish between different levels of P in the diet. The reason that no differences among sources were detected is that the differences in availability among the sources were too small to be detected by the scanning procedures.

Correlations between the scanning procedures and breaking strength were between 0.70 and 0.77 for the eight best DXA measurements. Likewise, correlation

between breaking strength and the six highest pQCT measurements were between 0.72 and 0.79. Interestingly, the polar strength strain indices were only correlated to breaking strength at 0.58. The polar strength strain indices are a calculated value being used to predict breaking strength in humans. Based on the current data, this value seems to be less accurate than some of the other measurements. The highest R^2 for the DXA scan was for global BMD and global BMC. Lochmüller et al. (2000) reported BMC in DXA to be correlated to femoral failure load with an R^2 from 0.54 - 0.67. Schiessl et al. (1996) found proximal and distal strength strain indices to be highly correlated ($R^2 = 0.93$) with muscle force, a measure of adaptation of bone to mechanical load. However, the principal components analysis revealed that global BMC explains most of the variation in the model. The prediction equations that were developed showed that the breaking strength may be predicted with a reasonable accuracy if 4 to 12 parameters from the scanning procedures are used. It also was shown that the data from the pQCT scan gave the best R^2 for the equation. This suggests that if prediction equations are used to predict breaking strength, then only the pQCT data are needed. The R^2 for the equations are not improved by adding the data from the DXA scan. The 50% slice BMC was the single parameter measured by pQCT that most precisely predicted breaking strength. Therefore, these data indicate that the mineral content of the bone may be the most important of the scanned measurements because it gives the best correlations with the breaking strength of a bone. This is true regardless of the procedure (i. e., DXA or pQCT) used to measure BMC. The results of the correlations between the BMC data and breaking strength also indicate that these measurements are as precise in predicting

breaking strength as are the prediction equations. The BMC is measured as the amount of P in the bone. Therefore, this agrees with the observation that the amount of ash has a higher correlation with breaking strength than has the concentration of bone ash.

Implications

The availability of P in MCP and DCP relative to MSP may be lower than demonstrated in previous reports, with DCP having the lowest availability. However, there are no differences in P-availability between MCP sources. Procedures for bone density scanning can be used to predict bone breaking strength with two advantages over bone breaking strength; the pig does not have to be harvested, and multiple measurements can be made over time. The best measurement for predicting bone breaking strength is BMC regardless of the type of scanning machine used (DXA or pQCT). Prediction equations can also be used to estimate bone breaking strength with reasonable accuracy.

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Table 5.1. Ingredient composition (%) of experimental diets (as is basis)

Item Diet:	1	2	3	4	5	6	7	8	9	10	11
Source:	Basal	DCP[a]	DCP[a]	MCP70[a]	MCP70[a]	MCP85[a]	MCP85[a]	MCP100[a]	MCP100[a]	MSP[a]	MSP[a]
Casein	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Corn	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Cornstarch	51.27	50.87	50.48	50.83	50.42	50.85	50.43	50.87	50.48	50.76	50.26
Sugar	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Potato prot.[b]	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Solka floc[c]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	0.23	0.26	0.28	0.30	0.36	0.32	0.41	0.34	0.44	0.47	0.70
DCP[a]	-	0.37	0.74	-	-	-	-	-	-	-	-
MCP70[a]	-	-	-	0.37	0.74	-	-	-	-	-	-
MCP85[a]	-	-	-	-	-	0.33	0.66	-	-	-	-
MCP100[a]	-	-	-	-	-	-	-	0.29	0.58	-	-
MSP[a]	-	-	-	-	-	-	-	-	-	0.27	0.54
L-Lys HCl	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14

DL-Met	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
L-Thr	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
L-Trp	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
K_2CO_3	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
MgO_2	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamins [d]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Minerals [e]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25

[a] DCP= dicalcium phosphate, MCP70= monocalcium phosphate, monocalcium phosphate with 70% purity, MCP85= monocalcium phosphate with 85% purity, MCP100= monocalcium phosphate with 100% purity, MSP= monosodium phosphate

[b] Potato Protein concentrate, Avebe America Inc., Princeton, NJ.

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

[d] The vitamin premix provided per kg of diet: 10,032 IU of vitamin A acetate; 992 IU of vitamin D_3 as d-activated animal sterol; 88 IU of vitamin E as alphatocopherol acetate; 1.5 mg of vitamin K as menadione dimethypyrimidinol bisulfate; 0.4 mg of biotin; 60 mg of niacin; 25 mg of pantothenic acid; 10mg of riboflavin; and 0.05 mg of vitamin B_{12}.

[e] The trace mineral premix provided per kg of diet: Cu, 23 mg as copper sulfate; Fe, 110 mg as iron sulfate; I, 0.275 mg as potassium iodate; Mn, 23 mg as manganese sulfate; Se, 0.275 mg as sodium selenite; Zn, 114 mg as zinc oxide.

Table 5.2. Analyzed nutritional composition of experimental diets (as is basis)

Ingredient (%):	Diet:	1	2	3	4	5	6	7	8	9	10	11
	Source:	Basal	DCP[a]	DCP[a]	MCP70[a]	MCP70[a]	MCP85[a]	MCP85[a]	MCP100[a]	MCP100[a]	MSP[a]	MSP[a]
Mcal ME/ kg[b]		3,790	3,773	3,758	3,772	3,754	3,773	3,756	3,774	3,758	3,769	3,750
Crude Protein, %		12.68	12.71	12.86	12.83	12.84	13.79	13.61	12.68	12.70	13.01	12.65
Calcium, %		0.13	0.20	0.30	0.21	0.30	0.19	0.28	0.20	0.27	0.19	0.28
Phosphorus, %		0.12	0.18	0.25	0.17	0.23	0.17	0.24	0.18	0.23	0.20	0.26
Lysine, %		0.92	1.03	1.07	0.94	0.85	0.88	0.91	0.93	0.97	0.96	0.93
Methionine, %		0.48	0.55	0.57	0.52	0.47	0.49	0.50	0.51	0.52	0.53	0.51
SAA[c], %		0.58	0.66	0.69	0.63	0.57	0.59	0.61	0.61	0.63	0.64	0.62
Threonine, %		0.49	0.56	0.60	0.52	0.45	0.48	0.49	0.51	0.52	0.52	0.50
Tryptophan, %		0.15	0.18	0.20	0.21	0.21	0.20	0.19	0.15	0.14	0.15	0.15
Histidine, %		0.28	0.32	0.34	0.29	0.25	0.27	0.27	0.28	0.30	0.29	0.28
Isoleucine, %		0.58	0.66	0.71	0.61	0.53	0.57	0.57	0.60	0.62	0.62	0.58
Leucine, %		1.15	1.33	1.41	1.21	1.05	1.13	1.13	1.20	1.23	1.23	1.16
Valine, %		0.73	0.84	0.89	0.74	0.65	0.69	0.70	0.75	0.75	0.76	0.71

Phenylalanine, %	0.65	0.74	0.79	0.68	0.61	0.64	0.64	0.67	0.69	0.69	0.65
Arginine, %	0.51	0.58	0.62	0.54	0.48	0.50	0.51	0.55	0.55	0.55	0.52

[a] DCP= dicalcium phosphate, MCP70= monocalcium phosphate, monocalcium phosphate with 70% purity, MCP85= monocalcium phosphate with 85% purity, MCP100= monocalcium phosphate with 100% purity, MSP= monosodium phosphate

[b] Values are calculated rather than analyzed

[c] Sulfur containing amino acids (methionine + cysteine)

Table 5.3. Effect of diet on pig performance and blood chemistry[a]

Diet:	Response : Added Phosphorus, (%):	Average daily P intake, g	Average daily gain, g	Average daily feed intake, g	Feed efficiency, G:F	Serum Ca, %	Serum P, %	Serum Ca:P
Basal	0	1.13	298	955	0.32	72.0	88.3	0.82
DCP[b]	0.07	1.85	330	1040	0.32	77.6	90.0	0.87
DCP[b]	0.14	2.75	393	1100	0.36	86.1	116.5	0.75
MCP70[b]	0.07	1.79	331	1036	0.32	82.7	92.7	0.88
MCP70[b]	0.14	2.92	413	1280	0.33	86.9	130.8	0.67
MCP85[b]	0.07	1.92	365	1138	0.32	84.1	99.8	0.84
MCP85[b]	0.14	2.74	408	1130	0.36	80.0	130.3	0.61
MCP100[b]	0.07	2.04	408	1123	0.36	82.0	112.5	0.73

MCP100[b]	0.14	2.61	369	1118	0.32	85.1	127.6	0.67
MSP[b]	0.07	2.22	350	1095	0.32	80.9	106.3	0.76
MSP[b]	0.14	2.97	398	1148	0.34	74.5	113.5	0.68
Pooled SE		0.14	38.37	67.20	0.03	4.48	7.62	0.05
P source		$P = 0.21$	$P = 0.96$	$P = 0.62$	$P = 0.99$	$P = 0.001$	$P = 0.04$	$P = 0.09$
P level		$P < 0.0001$	$P = 0.13$	$P = 0.12$	$P = 0.52$	$P = 0.51$	$P < 0.0001$	$P < 0.0001$
Source x level		$P = 0.38$	$P = 0.52$	$P = 0.31$	$P = 0.51$	$P = 0.0009$	$P = 0.08$	$P = 0.17$

[a] Data are means of four observations per diet

[b] DCP= dicalcium phosphate, MCP70= monocalcium phosphate with 70% purity, MCP85= monocalcium phosphate with 85% purity, MCP100= monocalcium phosphate with 100% purity, MSP= monosodium phosphate

Table 5.4. Effect of P-source on pig performance and blood chemistry[a]

Response: Phosphorus source:	Phosphorus feed intake, g	Average daily gain, g	Average daily feed intake, g	Feed efficiency, G:F	Serum Ca, %	Serum P, %	Serum Ca:P
Basal	1.13^{y}	298	955^{y}	0.32	72.0^{y}	88.3^{z}	0.82
DCP[b]	2.30^{x}	361	1070^{xy}	0.34	81.9^{xy}	103.2^{yz}	0.81
MCP70[b]	2.44^{x}	376	1176^{x}	0.32	85.4^{x}	115.1^{xy}	0.76
MCP85[b]	2.33^{x}	386	1134^{x}	0.34	82.0^{xy}	115.0^{xy}	0.73
MCP100[b]	2.33^{x}	385	1120^{xy}	0.34	83.8^{x}	121.3^{x}	0.70
MSP[b]	2.60^{x}	376	1121^{xy}	0.33	77.3^{xy}	108.7^{xy}	0.72
SEM	0.18	25.92	48.36	0.02	2.94	6.19	0.04
P-value	0.05	0.05	0.05	0.05	0.05	0.05	0.05

[a] Data are means of four observations for the basal diet and eight observations for all sources of P

[b] DCP= dicalcium phosphate, MCP70= monocalcium phosphate with 70% purity, MCP85= monocalcium phosphate with 85% purity, MCP100= monocalcium phosphate with 100% purity, MSP= monosodium phosphate

[x, y, z] Means within a column lacking a common superscript are different ($P < 0.05$)

Table 5.5. Influence of treatment on bone ash and bone breaking parameters[a]

Diet:	Response:	Metacarpal ash, %	Metacarpal ash, g	Metacarpal breaking strength, kg	Total bone Ca, %	Total bone P, %	Total bone Ca:P	Total bone Ca, g	Total bone P, g
	Added Phosphorus, (%):								
Basal	0	45.13	2.73	18.40	32.50	16.55	1.96	0.89	0.45
DCP[b]	0.07	48.32	2.86	27.88	32.16	16.61	1.94	0.92	0.48
DCP[b]	0.14	51.01	3.32	30.41	31.28	16.66	1.88	1.04	0.53
MCP70[b]	0.07	50.16	3.05	29.46	32.01	16.34	1.96	0.97	0.50
MCP70[b]	0.14	51.86	3.66	35.22	31.90	17.13	1.86	1.17	0.63
MCP85[b]	0.07	48.51	3.02	28.63	32.59	16.93	1.93	0.98	0.51
MCP85[b]	0.14	51.69	3.48	34.75	32.29	17.40	1.86	1.12	0.61
MCP100[b]	0.07	48.83	3.13	26.03	32.59	17.15	1.90	1.02	0.54
MCP100[b]	0.14	51.98	3.66	41.25	32.53	17.37	1.87	1.19	0.63
MSP[b]	0.07	50.46	3.47	35.28	32.48	17.23	1.89	1.12	0.60

MSP[b]	0.14	50.64	3.18	40.84	31.97	17.18	1.87	1.02	0.55
Pooled SE		0.85	0.13	3.04	0.44	0.15	0.02	0.07	0.04
P source		$P = 0.28$	$P = 0.02$	$P = 0.001$	$P = 0.003$	$P = 0.0001$	$P = 0.46$	$P = 0.20$	$P = 0.19$
P level		$P < 0.0001$	$P < 0.0001$	$P = 0.08$	$P = 0.008$	$P = 0.05$	$P < 0.0001$	$P = 0.009$	$P = 0.002$
Source x level		$P = 0.04$	$P = 0.0005$	$P = 0.30$	$P = 0.43$	$P = 0.08$	$P = 0.31$	$P = 0.08$	$P = 0.08$

[a] Data are means of four observations per diet

[b] DCP= dicalcium phosphate, MCP70= monocalcium phosphate with 70% purity, MCP85= monocalcium phosphate with 85% purity, MCP100= monocalcium phosphate with 100% purity, MSP= monosodium phosphate

Table 5.6. Effect of P-source on bone ash and bone breaking parameters[a]

Response: Phosphorus source:	Metacarpal ash, %	Metacarpal ash, g	Metacarpal breaking strength, kg	Total bone Ca, %	Total bone P, %	Total Ca:P	Total bone Ca, g	Total bone P, g
Basal	45.13^{y}	2.73^{y}	18.40^{z}	32.50^{x}	16.55^{y}	1.96^{x}	0.89^{y}	0.45^{y}
DCP[b]	49.67^{x}	3.09^{y}	29.14^{y}	31.72^{y}	16.64^{y}	1.91^{y}	0.98^{xy}	0.51^{xy}
MCP70[b]	51.19^{x}	3.40^{x}	32.75^{xy}	31.90^{xy}	16.79^{y}	1.91^{y}	1.08^{x}	0.57^{x}
MCP85[b]	50.10^{x}	3.25^{x}	31.69^{xy}	32.44^{x}	17.16^{x}	1.89^{y}	1.05^{xy}	0.56^{x}
MCP100[b]	50.51^{x}	3.39^{x}	33.64^{xy}	32.48^{x}	17.26^{x}	1.88^{y}	1.10^{x}	0.59^{x}
MSP[b]	50.44^{x}	3.32^{x}	38.06^{x}	32.31^{xy}	17.21^{x}	1.88^{y}	1.07^{x}	0.57^{x}
SEM	0.58	0.11	2.50	0.22	0.12	0.02	0.05	0.03
P-value	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

[a] Data are means of four observations the basal diet and eight observations for all sources of P

[b] DCP= dicalcium phosphate, MCP70= monocalcium phosphate with 70% purity, MCP85= monocalcium phosphate with 85% purity, MCP100= monocalcium phosphate with 100% purity, MSP= monosodium phosphate

[x, y, z] Means within a column lacking a common superscript are different ($P < 0.05$)

Table 5.7. Influence of treatment on Dual-energy X-ray Absorptiometry (DXA) bone density parameters[a]

Diet:	Response: Added Phosphorus, (%):	Global bone mineral content, g	Global bone mineral density, g/cm^3	R1[b] bone mineral density, g/cm^3	R3[b] bone mineral content, g	R4[b] bone mineral content, g	Polar strength strain indices 50%[c]
Basal	0	4.77	0.25	0.19	9.93	0.91	178.65
DCP[d]	0.07	6.08	0.30	0.24	1.10	1.06	191.88
DCP[d]	0.14	6.74	0.32	0.26	1.27	1.24	224.67
MCP70[d]	0.07	5.82	0.30	0.24	1.15	1.11	211.65
MCP70[d]	0.14	7.79	0.35	0.30	1.40	1.45	252.67
MCP85[d]	0.07	6.17	0.30	0.22	1.13	1.05	212.66
MCP85[d]	0.14	7.55	0.37	0.30	1.33	1.30	219.15
MCP100[d]	0.07	6.24	0.31	0.24	1.18	1.11	193.82
MCP100[d]	0.14	8.38	0.40	0.33	1.54	1.51	243.73

MSP[d]	0.07	6.67	0.32	0.24	1.33	1.31	226.31
MSP[d]	0.14	7.07	0.35	0.30	1.32	1.26	223.93
Pooled SE		0.51	0.02	0.02	0.10	0.08	19.92
P source		$P = 0.35$	$P = 0.18$	$P = 0.46$	$P = 0.25$	$P = 0.08$	$P = 0.64$
P level		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P = 0.002$	$P < 0.0001$	$P = 0.03$
Source x level		$P = 0.23$	$P = 0.14$	$P = 0.27$	$P = 0.28$	$P = 0.04$	$P = 0.46$

[a] Data are means of four observations per diet

[b] R1= 0-20% of the total tibia length from at the distal end, R3= 40-60% of total tibia length from the distal end, R4= 60-80% of total tibia length from the distal end

[c] Polar strength strain indices= $\sum_{i=1n} r_i^2 * a \frac{CD}{ND}$

where r= distance of a voxel from the center of gravity, r_{max}= maximum distance of a voxel from the center of gravity, a= area of a voxel (mm^2), CD= measured cortical density (mg/cm), ND= normal physiological density (1200 mg/cm^3)

[d] DCP= dicalcium phosphate, MCP70= monocalcium phosphate with 70% purity, MCP85= monocalcium phosphate with 85% purity, MCP100= monocalcium phosphate with 100% purity, MSP= monosodium phosphate

Table 5.8. Effect of P-source on Dual-energy X-ray Absorptiometry (DXA) bone density parameters[a]

Response: / Phosphorus source:	Global bone mineral content, g	Global bone mineral density, g/cm^3	R1 bone mineral density, g/cm^3	R3 bone mineral content, g	R4 bone mineral content, g	Polar strength strain indices 50%[c]
Basal	4.77^{y}	0.25^{y}	0.19^{y}	0.93^{y}	0.91^{y}	178.65^{y}
DCP[b]	6.40^{x}	0.31^{x}	0.25^{x}	1.19^{xy}	1.15^{xy}	208.27^{xy}
MCP70[b]	6.94^{x}	0.33^{x}	0.27^{x}	1.29^{x}	1.30^{x}	235.15^{x}
MCP85[b]	6.86^{x}	0.34^{x}	0.26^{x}	1.23^{x}	1.17^{x}	215.91^{xy}
MCP100[b]	7.31^{x}	0.35^{x}	0.28^{x}	1.36^{x}	1.31^{x}	218.89^{xy}
MSP[b]	6.87^{x}	0.33^{x}	0.27^{x}	1.33^{x}	1.29^{x}	225.01^{xy}
SEM	0.43	0.01	0.02	0.08	0.07	14.31
P-value	0.05	0.05	0.05	0.05	0.05	0.05

[a] Data are means of four observations the basal diet and eight observations for all sources of P

[b] DCP= dicalcium phosphate, MCP70= monocalcium phosphate with 70% purity, MCP85= monocalcium phosphate with 85% purity, MCP100= monocalcium phosphate with 100% purity, MSP= monosodium phosphate

[c] R1= 0-20% of the total tibia length from at the distal end, R3= 40-60% of total tibia length from the distal end, R4= 60-80% of total tibia length from the distal end

[d] Polar strength strain indices= $\sum_{i=1n} r_i^2 * a \frac{CD}{ND}$ / r_{max}

where r= distance of a voxel from the center of gravity, r_{max}= maximum distance of a voxel from the center of gravity, a= area of a voxel (mm^2), CD= measured cortical density (mg/cm), ND= normal physiological density (1200 mg/cm^3)

[x, y] Means within a column lacking a common superscript are different ($P < 0.05$)

Table 5.9. Influence of treatment on Peripheral Quantitative Computed Tomography (pQCT) bone density parameters[a]

Diet:	Response:	Trabecular BMD 10%, mg/cm^3	Trabecular area 10%, mm^2	Cortical BMC 50%, mg/mm	Cortical thickness 50%, mm	Cortical area 50%, mm^2	Endosteal circ.[b] 50%, mm	Total BMC 50%, mg/mm	Cortical area 70%, mm^2
	Added Phosphorus, (%):								
Basal	0	136.22	519.95	39.84	1.11	42.50	37.19	61.85	68.81
DCP[c]	0.07	132.28	471.69	50.50	1.32	52.56	34.35	71.29	69.30
DCP[c]	0.14	158.32	509.96	55.38	1.44	58.01	31.48	78.84	68.31
MCP70[c]	0.07	149.60	493.70	51.35	1.35	53.19	32.66	73.83	69.45
MCP70[c]	0.14	168.80	508.39	66.72	1.65	66.06	35.43	83.02	62.78
MCP85[c]	0.07	151.95	507.69	52.91	1.44	55.69	34.49	74.76	68.09
MCP85[c]	0.14	178.37	482.06	66.01	1.75	67.12	33.14	85.19	68.40
MCP100[c]	0.07	151.22	465.43	51.20	1.35	52.97	34.54	70.36	64.46

MCP100[c]	0.14	182.05	514.97	75.23	2.00	75.35	32.94	91.63	68.15
MSP[c]	0.07	158.92	563.85	58.37	1.51	59.54	33.31	79.10	62.78
MSP[c]	0.14	175.75	470.26	64.11	1.79	65.12	35.65	81.51	72.41
Pooled SE		5.17	44.20	4.88	0.12	4.60	1.63	4.88	3.91
P source		*P* = 0.01	*P* = 0.79	*P* = 0.20	*P* = 0.09	*P* = 0.27	*P* = 0.77	*P* = 0.64	*P* = 0.93
P level		*P* = 0.01	*P* = 0.75	*P* = 0.0001	*P* = 0.0001	*P* = 0.0002	*P* = 0.81	*P* = 0.0009	*P* = 0.58
Source x level		*P* = 0.71	*P* = 0.37	*P* = 0.20	*P* = 0.25	*P* = 0.25	*P* = 0.09	*P* = 0.28	*P* = 0.39

[a] Data are means of four observations per diet

[b] Circumference

[c] DCP= dicalcium phosphate, MCP70= monocalcium phosphate with 70% purity, MCP85= monocalcium phosphate with 85% purity, MCP100= monocalcium phosphate with 100% purity, MSP= monosodium phosphate

Table 5.10. Effect of P-source on Peripheral Quantitative Computed Tomography (pQCT) bone density parameters[a]

Response: Phosphorus source:	Trabecular density 10%, mg/cm^3	Trabecular area 10%, mm^2	Cortical mineral content 50%, mg/mm	Cortical thickness 50%, mm	Cortical area 50%, mm^2	Endosteal circ[b] 50%, mm	Total mineral content 50%, mg/mm	Cortical area 70%, mm^2
Basal	159.85	438.86^{y}	39.84^{y}	1.11^{y}	42.50^{x}	35.13^{xy}	61.85^{y}	59.85^{y}
DCP[c]	157.64	472.78^{y}	52.94^{xy}	1.38^{xy}	55.28^{xy}	35.69^{x}	75.07^{xy}	68.25^{xy}
MCP70[c]	158.79	465.83^{y}	60.13^{x}	1.52^{x}	60.54^{x}	35.14^{xy}	79.08^{x}	65.78^{xy}
MCP85[c]	157.09	502.18^{y}	59.46^{x}	1.59^{x}	61.40^{x}	33.70^{xy}	79.98^{x}	72.08^{x}
MCP100[c]	156.25	506.07^{y}	63.21^{x}	1.67^{x}	64.16^{x}	33.14^{xy}	81.00^{x}	68.00^{xy}
MSP[c]	163.21	581.33^{x}	61.24^{x}	1.65^{x}	62.33^{x}	32.81^{y}	80.30^{x}	66.97^{xy}
Pooled SE	6.87	21.92	4.14	0.11	3.84	0.92	3.87	2.52
P source	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

[a] Data are means of four observations the basal diet and eight observations for all sources of P

[b] Circumference

[c] DCP= dicalcium phosphate, MCP70= monocalcium phosphate with 70% purity, MCP85= monocalcium phosphate with 85% purity, MCP100= monocalcium phosphate with 100% purity, MSP= monosodium phosphate

[x, y, z] Means within a column lacking a common superscript are different ($P < 0.05$)

Table 5.11. Correlation between breaking strength and scanned measures for bone density

Bone density parameter	Correlation to metacarpal bone breaking strength	*P*-value
DXA parameters		
Global bone mineral density, g/cm^3	0.77	< 0.0001
Global bone mineral content, g	0.75	< 0.0001
R4 bone mineral content[a], g	0.75	< 0.0001
R1 bone mineral density [a], g/cm^3	0.73	< 0.0001
R3 bone mineral content [a], g	0.73	< 0.0001
R4 bone mineral density [a], g/cm^3	0.72	< 0.0001
R3 bone mineral density [a], g/cm^3	0.70	<0.0001
R2 bone mineral density [a], g/cm^3	0.70	<0.0001
pQCT parameters		
Cortical area 50%, mm^2	0.79	< 0.0001
Total mineral content of bone 50%, g	0.79	< 0.0001
Cortical mineral content 50%, g	0.78	< 0.0001
Cortical thickness 50%, mm	0.77	< 0.0001
Total mineral density 50%, g/cm^3	0.72	<0.0001
Cortical mineral content 70%, g	0.72	<0.0001
Polar strength strain indices[b] 50%	0.58	< 0.0001

[a] R1= 0-20% of the total tibia length from at the distal end, R2= 20-40% of total tibia length from the distal end, R3= 40-60% of total tibia length from the distal end, R4= 60-80% of total tibia length from the distal end

[b] Polar strength strain indices= $\sum_{i=1n} r_i^2 * a \frac{CD}{ND}$ / r_{max}

where r= distance of a voxel from the center of gravity, r_{max}= maximum distance of a voxel from the center of gravity, a= area of a voxel (mm^2), CD= measured cortical density (mg/cm), ND= normal physiological density (1200 mg/cm^3)

Table 5.12. Proportion of variation in the model explained by Peripheral Quantitative Computed Tomography (pQCT) and Dual-Energy X-ray Absorptiometry (DXA)

Density parameter	Proportion of variation explained by a bone density parameter	Cumulative proportion of variation explained
Global bone mineral content, g	53.09	53.09
Endosteal circumference 50%, mm	17.48	70.57
R1 area[a], mm^2	7.38	77.94
Trabecular area 10%, mm^2	6.29	84.23
Trabecular density 10%, g/cm^3	3.99	88.22
Cortical area 70%, mm^2	2.37	90.59
Polar area moment of inertia of the area[b]	2.23	92.81
R2 bone mineral density[a], g/cm^3	1.52	94.33

[a] R1= 0-20% of the total tibia length from at the distal end, R2= 20-40% of total tibia length from the distal end, R3= 40-60% of total tibia length from the distal end, R4= 60-80% of total tibia length from the distal end

[b] Polar area moment of inertia of the area [mm*4] =$\sum$a* ((mean x-coordinate of all voxels of the cortical area- x)^2 + (mean y-coordinate of all voxels of the cortical area- y)^2) a= area of one voxel, sum is performed over the cortical area

Table 5.13. Proportion of variation in the model explained by Dual-Energy X-ray Absorptiometry (DXA)

Density parameter	Proportion of variation explained by a bone density parameter	Cumulative proportion of variation explained
Global bone mineral content, g	64.88	64.88
R2 bone mineral density[a], g/cm^3	18.51	83.39
R5 area[a], mm^2	4.45	87.84
R1 bone mineral density[a], g/cm^3	4.03	91.87
R5 bone mineral content[a], g	2.86	94.72

[a] R1= 0-20% of the total tibia length from at the distal end, R2= 20-40% of total tibia length from the distal end, R5= 80-100% of total tibia length from the distal end

Table 5.14. Proportion of variation in the model explained by Peripheral Quantitative Computed Tomography (pQCT)

Density parameter	Proportion of variation explained by a bone density parameter	Cumulative proportion of variation explained
Total mineral content 50%, g	50.52	50.52
Endosteal circumference 50%, mm	21.65	72.17
Trabecular area 10%, mm^2	9.13	81.30
Endosteal circumference 70%, mm	7.46	88.76
Cortical density 70%, g/cm^3	3.76	92.52
Trabecular density 10%, g/cm^3	2.89	95.41

Table 5.15. Prediction of bone breaking strength from all analyzed bone density parameters

	Equation	R^2
Both instruments	y = (Periosteal circumference, 10% slice * 0.45) + (Total area, 70% slice * -0.16) + (Cortical mineral density, 70% slice * -0.07) + (Cortical area, 70% slice * -0.80) + (Total mineral content, 50% slice * -2.86) + (Total mineral density, 50% slice * 0.40) + (Total area, 50% slice * -5.87) + (Cortical thickness, 50% slice * 325.83) + (Endosteal circumference, 50% slice * 43.16) + (Polar moment of resistance of the cortical area, 50% slice * 0.29) + (Moment of inertia standardized weight displacement in the bone, 50% slice * 0.03) + (Polar strength strain indices[c], 50% slice * -0.37) -1050.77	0.85
pQCT[a]	y = (Periosteal circumference, 10% slice * 0.45) + (Total area, 70% slice * -0.16) + (Cortical mineral density, 70% slice * -0.07) + (Cortical area, 70% slice * -0.80) + (Total mineral content, 50% slice * -2.86) + (Total mineral density, 50% slice * 0.40) + (Total area, 50% slice * -5.87) + (Cortical thickness, 50% slice * 325.83) + (Endosteal circumference, 50% slice * 43.16) + (Polar moment of resistance of the cortical area, 50% slice * 0.29) + (Moment of inertia standardized weight displacement in the bone, 50% slice *	0.85

	0.03) + (Polar strength strain indices[c], 50% slice * -0.37) -1050.77	
DXA[b]	y = (Global bone mineral density * 99.82) + (R4 bone mineral density[d] * 114.51) + (R5 area[d] * 29.32) + (R5 bone mineral content[d] * -76.90) + (R5 bone mineral density[d] * 392.46) -186.56	0.70
Maximum of four parameters		
Both instruments	y = (Total mineral content, 50% slice * -2.86) + (Total mineral density, 50% slice * 0.40) + (Cortical thickness, 50% slice * 325.83) + (Moment of inertia standardized weight displacement in the bone, 50% slice * 0.03) -84.01	0.72
pQCT[a]	y = (Total mineral content, 50% slice * -2.86) + (Total mineral density, 50% slice * 0.40) + (Cortical thickness, 50% slice * 325.83) + (Moment of inertia standardized weight displacement in the bone, 50% slice * 0.03) -84.01	0.72
DXA[b]	y = (R4 bone mineral density[d] * 174.29) + (R5 area[d] * 37.37) + (R5 bone mineral content[d] * -96.65) + (R5 bone mineral density[d] * 544.82) -233.32	0.66

[a] Peripheral Quantitative Computed Tomography

[b] Dual-Energy X-ray Absorptiometry

[a] Polar strength strain indices= $\sum_{i=1n} r_i^2 * a \frac{CD}{ND}$ / r_{max}

where r= distance of a voxel from the center of gravity, r_{max}= maximum distance of a voxel from the center of gravity, a= area of a voxel (mm^2), CD= measured cortical density (mg/cm), ND= normal physiological density (1200 mg/cm^3)

[d] R4= 60-80% of total tibia length from the distal end, R5= 80-100% of total tibia length from the distal end

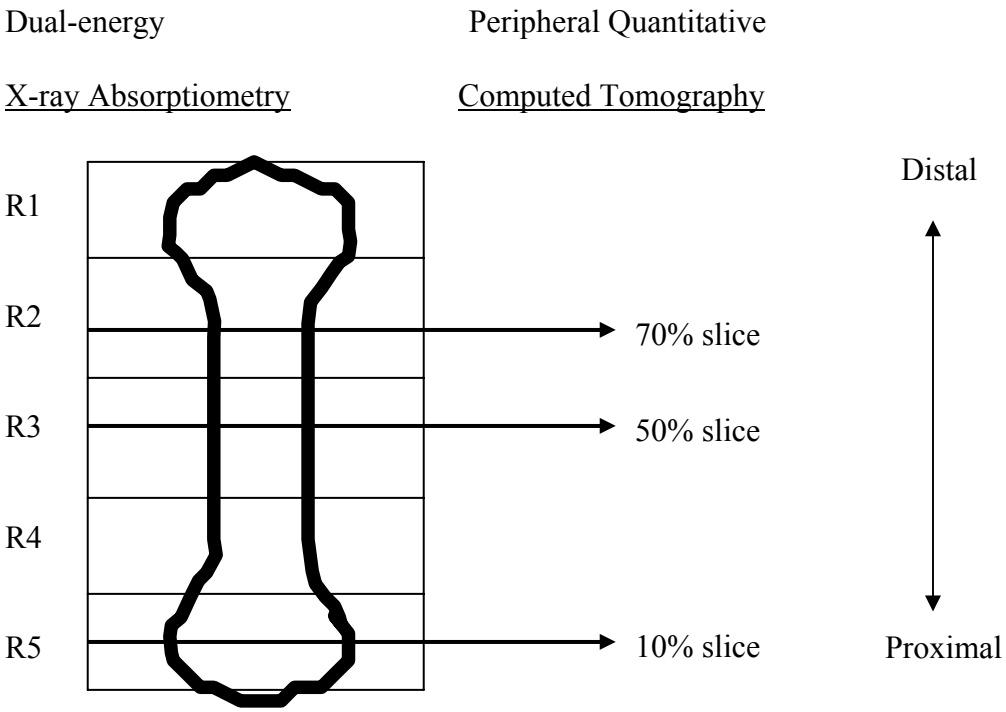

Figure 5.1. Illustration of areas of the bone measured by Dual-energy X-ray Absorptiometry and Peripheral Quantitative Computed Tomography

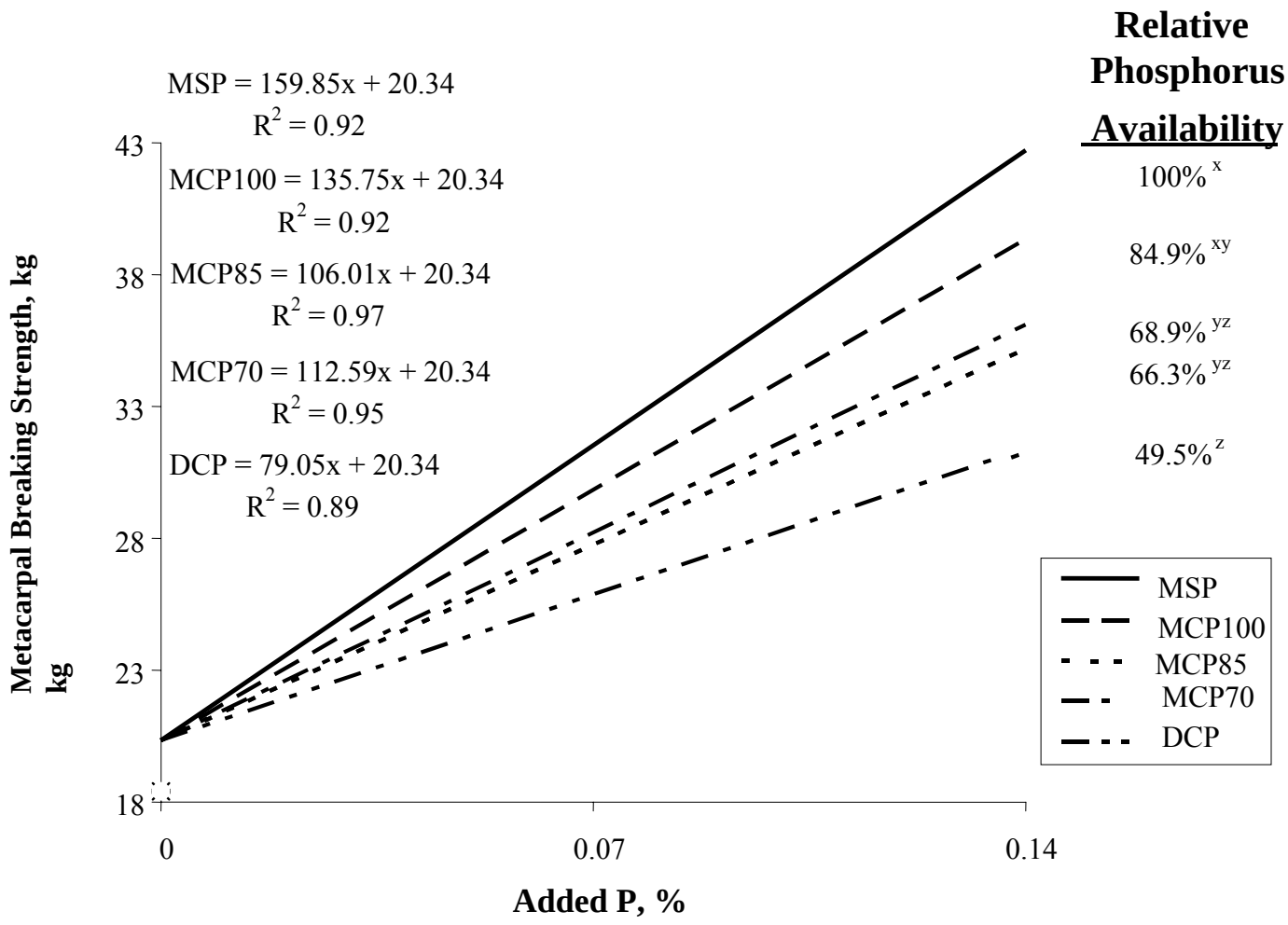

Figure 5.2. Relative Phosphorus Bioavailability in five sources of inorganic phosphorus

CHAPTER 6

Conclusion

From the three experiments that are reported in this thesis, it is concluded that the apparent (ATTD) and true total tract digestibility (TTTD) of P may be measured using the direct procedure. This procedure may be used to measure the digestibility of P in organic as well as inorganic P-sources. However, this requires the use of a P-free diet. The studies have shown that gelatin may be used as an amino acid (AA) source in such a diet provided that crystalline Ile, His, Met, and Trp are added to the diet to compensate for the low concentration of those AA in gelatin. In the first experiment, it was shown that the AA in such a diet have a digestibility comparable to soybean meal. This P-free diet can also be used to measure endogenous losses of P.

It was demonstrated that monosodium phosphate (MSP) is almost completely digested with a TTTD of above 98%. There were no differences in ATTD or TTTD between dicalcium phosphate (DCP) and monocalcium phosphate (MCP) and there were no differences among these different sources of MCP.

The ATTD and TTTD that were found using the above procedure were compared to data for metacarpal bone breaking strength that were obtained using a slope-ratio technique. This technique confirmed that P in MSP is more available than P in the other P-sources. It was also found that there were no differences among MCP sources. However, DCP had a lower P-availability than MSP and one of the MCP sources.

The data obtained using the slope-ratio procedure were compared to data for bone density that were generated using either a dual-energy X-ray absorbitometry (DXA) or a

peripheral quantitative computed tomography scan. It was shown that metacarpal breaking strength can be predicted using bone mineral content and bone mineral density measures in either scanning device. Prediction equations were also formulated to estimate bone breaking strength using data from the scanning procedures. Therefore, bone breaking strength may be predicted from bone density measures.

In conclusion, it is possible to utilize a P-free diet for the direct calculation of P-digestibility in inorganic sources of P. The current data also demonstrate that it is also possible to evaluate different P-sources by measuring bone density in pigs fed these sources. This will allow swine producers to more accurately formulate diets that satisfy the pigs' requirement without overfeeding phosphorus. Therefore, the amount of P excreted into the environment from pigs will be reduced if this procedure is used.