ASPECTS OF PHOSPHORUS NUTRITION IN SWINE

BY

SCOTT R. BAKER

THESIS

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Professor Hans Henrik Stein

ABSTRACT

Aspects of Phosphorus Nutrition in Swine

Scott R. Baker

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Three experiments were conducted to evaluate aspects of phosphorus nutrition in pigs. The first 2 experiments were conducted to determine the proper methodology for determining the utilization of P in pigs. The relative bioavailability procedure that uses a slope ratio method was compared with the total tract digestibility method. The 2 experiments were carried out to compare the relative bioavailability (**RBV**) values of P in dicalcium phosphate (**DCP**) and distillers dried grains with solubles (**DDGS**) with the apparent total tract digestibility (**ATTD**) and the standardized total tract digestibility (**STTD**) of the same ingredients. In Exp 1, eighteen growing pigs (initial BW; 34.93 ± 1.04 kg) were allotted to 3 cornstarch-based diets and housed individually in metabolism cages. One diet was a P-free diet that was used to determine endogenous P losses (**EPL**). Two additional diets in which DCP or DDGS was the sole source of P were also formulated. Results showed that the ATTD of P in DCP and DDGS were 86.1 and 58.8%, respectively. The EPL was determined to be 174 mg/kg DMI and the STTD of P was calculated to be 93.1% in DCP and 63.1% in DDGS. In Exp 2, forty two pigs (initial BW: 29.02 ± 2.03 kg) were allotted to 7 diets and housed individually. A basal diet (0.22% P) was formulated and 3 additional diets were formulated by adding 0.04, 0.08, or 0.12% P from DCP to the basal diet. The remaining 3 diets were formulated by adding 0.04, 0.08, or 0.12% P from

DDGS to the basal diet at the expense of cornstarch. The animals were fed their respective diets for 28 d and they were then euthanized. The third and fourth metacarpals were removed from the front right foot of the animals. Metacarpal bone ash and bone P were regressed against P intake for each ingredient using the slope ratio method. It was determined that the bioavailability of P in DDGS was 60% relative to that in DCP. It was concluded that there was good agreement between values for RBV and STTD values. In Exp 3, the effect of BW on EPL and nutrient digestibility was determined. A semi-purified P-free diet was formulated to determine EPL. Three additional diets containing soybean meal (**SBM**), DDGS, and corn as sole source of P were also formulated. Twenty-four barrows (initial BW: 9.66 ± 0.67 kg) were allotted to the 4 diets and placed in metabolism cages for five 12-d collection periods. Pigs had an average BW of 9.66 ± 0.67, 22.29 ± 2.57, 53.77 ± 9.91, 92.73 ± 16.17, and 129.23 ± 18.55 kg at the start of each collection period. Pigs were fed the same diet during all collection periods, but between collection periods they were fed a corn-soybean meal-DDGS diet. During the collection periods fecal samples were collected quantitvely from d 6 to 11 and values for ATTD, EPL, and STTD of P and ATTD of Ca, GE, CP, ether extract, ADF, NDF, and ash were calculated. There was no effect of BW on EPL and the average EPL was 220 mg/kg DMI. The ATTD and STTD of P in SBM and corn increased (linear, $P < 0.01$) as the BW of pigs increased, but that was not the case for DDGS. For the SBM diet, there was an increase in the ATTD of CP (linear and quadratic, $P < 0.05$), ether extract, GE, and ash (quadratic, $P < 0.05$) as pig BW increased, but there was no effect ($P > 0.05$) of BW on the ATTD of ADF, NDF, and Ca. For the DDGS diet, there was an increase in the ATTD of GE, NDF, and CP (linear and quadratic, $P < 0.05$), ether extract, and ADF (linear, $P < 0.05$), and of ash (quadratic, $P < 0.01$) as BW increased, but the ATTD of Ca in DDGS decreased (linear, $P < 0.01$) as pig BW increased. For corn, there was an increase (linear

and quadratic, $P < 0.01$) in DE and in the ATTD of CP, ADF, and NDF (linear, $P < 0.05$) and of ash (linear and quadratic, $P < 0.01$) as pig BW increased, but there was no effect ($P > 0.05$) of pig BW on the ATTD of GE, ether extract, or Ca. It was concluded that the ATTD and STTD of P in corn and SBM, but not in DDGS, increases as the pig BW increases, but EPL of P is constant regardless of pig BW. The influence of pig BW on the ATTD of GE and nutrients is dependent on the diet.

Keywords: apparent digestibility, bioavailability, body weight, dicalcium phosphate, distillers dried grains with solubles, endogenous losses, phosphorus, pig, standardized digestibility

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LIST OF ABBREVIATIONS

%	Percent
±	Plus or minus
$1,25(OH)_2D_3$	1,25-dihydroxyvitamin D3
AA	Amino acid
Abstr	Abstract
ADF	Acid detergent fiber
ADFI	Average daily feed intake
ADP	Adenosine diphosphate
ADG	Average daily gain
Adv	Advances
Agric	Agriculture
Al	Aluminum
Ala	Alanine
AM	American
AMP	Adenosine monophosphate

Anim	Animal
Ann	Annual
ANOVA	Analysis of variance
AOAC	Association of Analytical Chemists
AR [1]	First order autoregressive
Arch	Archive
Arg	Arginine
Asp	Aspartic acid
ATP	Adenosine-5'-triphosphate
ATTD	Apparent total tract digestibility
Austr	Australia
Biochem	Biochemistry
Biol	Biology
Biophys	Biophysics
Br	British
B.S.	Bachelor of Science
BW	Body weight

^{o}C	Degrees Celsius
CA	California
Ca	Calcium
cal	Calorie
Can	Canadian
Chem	Chemistry
CP	Crude protein
CT	Calcitonin
Cu	Copper
Cys	Cysteine
d	Day
DCP	Dicalcium phosphate
DDGS	Distillers dried grains with solubles
DE	Digestible energy
Diss	Dissertation
DL-Met	DL-methionine
DM	Dry Matter

DMI	Dry matter intake
DNA	Deoxyribonucleic acid
Dr	Doctor
Ed	Edition
ed	Editor
EPL	Endogenous phosphorus losses
et al.	And others
Exp	Experiment
Fe	Iron
FL	Florida
FTU	Phytase units
g	Grams
G:F	Gain to feed ratio
GE	Gross energy
Glu	Glutamine
Gly	Glycine
h	Hour

$H_2PO_4^-$	Dihydrogen phosphate ion
His	Histidine
HPO_4^{-2}	Hydrogen phosphate ion
I	Iodine
i.e.	Id Est (That is)
IA	Iowa
IL	Illinois
Ile	Isoleucine
IML	Interactive matrix language
Inc	Incorporated
Inst	Institute
Int	International
ISRL	Import Swine Research Lab
IU	International units
J	Journal
Jr	Junior
Kcal	Kilocalories

kg	Kilograms
L	Linear
Leu	Leucine
L-His	L-histidine
L-Ile	L-isoleucine
LLC	Limited Liability Corporation
L-Lys-HCL	L-lysine-hydrochloric acid
L-Thr	L-threonine
L-Trp	L-Tryptophan
L-Val	L-valine
Lys	Lysine
m	meter
MA	Massachusetts
Mcal	Megacalories
MCP	Monocalcium phosphate
ME	Metabolizable energy
Met	Methionine

Mg	Magnesium
mg	Milligrams
mm	Millimeters
MN	Minnesota
Mn	Manganese
MO	Missouri
M.S.	Master of Science
MSP	Monosodium phosphate
Na+	Sodium
NC	North Carolina
NDF	Neutral detergent fiber
NJ	New Jersey
No	Number
NRC	National Research Council
Nutr	Nutritional
NY	New York
OH	Ohio

P	Phosphorus
P	*P*-value
PA	Pennsylvania
Pf	Fecal output of phosphorus
Ph.D	Doctor of Philosophy
Phe	Phenylalanine
Phys	Physics
Physiol	Physiology
Pi	Phosphorus intake
PO_4^{-3}	Phosphate ion
Pro	Proline
Prof	Professional
PTH	Parathyroid hormone
Q	Quadratic effect
RBV	Relative bioavailability
Res	Research
RNA	Ribonucleic acid

Rev	Review
SAS	Statistical analysis software
SBM	Soybean meal
Sci	Science
Se	Selenium
SEM	Standard error of the mean
Ser	Serine
SRC	Swine Research Center
STTD	Standardized total tract digestibility
Suppl	Supplement
Technol	Technology
Thr	Threonine
Trp	Tyrptophan
TTTD	True total tract digestibility
Tyr	Tyrosine
Univ	University
U.S.	United States of America

USA	United States of America
Val	Valine
Vol	Volume
Wt	Weight
Zn	Zinc
α	Alpha

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CHAPTER 1

INTRODUCTION

There are 15 essential minerals that must be taken into consideration when preparing swine diets (Richert, 2010). Although all minerals are important, much attention is currently being directed towards P, an abundant element that is available in many forms with the most common ion being HPO_4^{2-} (Anderson et al., 2006). Phosphorus is important for bone mineralization and membrane stability and structure. Phosphorus also provides the P backbone of RNA and DNA and is a part of phospholipids (Anderson et al., 2006). Additionally, P is involved in nearly every cell via the energy molecules AMP, ADP, and ATP (Bauman, 2004). Thus, P is required for many functions in the body and is vital for sustainability of life.

Phosphorus has a low bio-availability in most feedstuffs of plant origin (Cromwell, 1992). Therefore, P is supplemented to most swine diet. The undigested P from the vegetable ingredients is excreted by the animal causing high levels of P in the manure, which may result in environmental pollution (Gerritse and Zugec, 1977; Klopfenstein et al., 2002). Research has, therefore, been conducted to develop methods to better utilize the P in natural feedstuffs of vegetable origin, which is intended to minimize dietary P supplementation and reduce total P excretion from the animal. This may increase environmental stewardship and decrease the costs of the diets. With increased focus on both the environment and costs of swine diets it is imperative that nutritionists know proper inclusion levels of P in rations. Therefore, accurate digestibility values of P-containing feed ingredients must be determined.

Several methods to determine P utilization by animals have been proposed. Relative bio-availability procedures have been used for calculations of P availability. This method uses the

slope-ratio procedure and arbitrarily correlates P-availability in all ingredients to the availability of P in a standard source, which is often monosodium phosphate or dicalcium phosphate (Cromwell, 1992). Recently a novel procedure has been proposed using standardized total track digestibility (**STTD**) as a more accurate method of P digestibility calculation (Petersen and Stein, 2006; Almeida and Stein, 2010). This procedure involves measurement of values for apparent total tract digestibility (**ATTD**) of P as well as determination of basal endogenous losses of P. Values for STTD are calculated by correcting ATTD values for basal endogenous losses. A major advantage of the STTD method is that the STTD values in individual ingredients are believed to be additive in mixed diets. It is, therefore, believed that diets formulated based on values for the STTD of P will more accurately meet the requirements of the animals and at the same time minimize P excretion in the manure. There is, however, a need for conducting research to understand factors that affect the STTD values of P.

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CHAPTER 2

PHOSPHORUS IN SWINE NUTRITION: LITERATURE REVIEW

Introduction

Phosphorus, an essential mineral in swine diets, plays a vital role in numerous body functions. In the adult pig, only 25% of total P resides in the soft tissues (Reese et al., 2010). The remaining P is stored in the bones giving the animal structural strength and stability. The amounts of P intake that are needed to mineralize bone and to sustain maximal growth are different. In other words, if dietary P level meets maximal growth potential, the animal will not have enough P intake to reach its maximum bone mineralization capacity (Cromwell et al., 1970). It is important to determine the level of P intake needed to reach the maximum performance of the animal. To do this, it is important to realize the levels of P utilization in all ingredients as they vary greatly and P may also interact with other factors within the diet (Cromwell et al., 1972). In the following review, biological utilization of P, dietary considerations, common sources of P, and influencing factors will be discussed in an attempt to emphasize the importance of understanding accurate P availability in feed ingredients. Methods for calculating P availability, absorption, and digestibility will be discussed.

Biological Utilization of P

In the body of the animal there is an overall balance of P. This balance is between the P absorbed in the small intestine and the P excreted in the feces and urine. In a growing, gestating, or lactating animal one must take into consideration the P needed to support growth and production (Kemme et al., 1997). The skeletal structure of the animal serves as a medium where

P is stored (Anderson et al., 2006). There are several ways of studying P digestibility and absorption. To understand the concept of P digestibility it is important to understand absorption, metabolism, homeostasis, and excretion.

Mechanisms of P Absorption

Digestibility of P in feedstuffs can vary from 10 to 90% (Jongbloed et al., 1991). Feed ingredients of animal origin generally have greater P-digestibility than feedstuffs of plant origin. The difference in digestibility between plant and animal sources is mainly caused by the form P is stored in ingredients. In commonly fed grains, most P is bound in an organic complex called the phytate complex that is indigestible to the monogastric animal (Cromwell, 1992). To digest this complex, enzymatic treatment or further processing is required to change the conformation of the inositol ring structure that renders the phytic acid molecule undigestible (Rimbach and Pallauf, 1997) in the small intestine, because pigs do not secrete enzymatic phytase along the brush border or in their saliva. Because P is absorbed in the small intestine as inorganic P, most of the natural P present in plant sources is not utilized. Using labeled P^{32}, it was observed that P is digested primarily in the upper small intestine (Moore and Tyler, 1955). Limited P absorption in the large bowel results in excretory losses for any P that is not digested in the small intestine (Jongbloed, 1987). Therefore, it is important that P is in a readily absorbable form when it reaches the small intestine so maximal absorption can occur.

The predominant form of absorbed P is the inorganic anion, specifically PO_4^- , $H_2PO_4^-$, and HPO_4^-. When inorganic P enters the small intestine it is rapidly taken up by the epithelial cells along the lumen of the brush-border membrane via active transport or passive diffusion (Anderson, 1991). Phosphorus is actively transported across the negative concentration gradient

until equilibrium is achieved (Peterlik and Wasserman, 1978). The Na^+ dependent process relies on intracellular sodium levels in the lumen in addition to vitamin-D and ATP (Lee et al.,1986 a). This system is easily overwhelmed and is used primarily during times of low P levels in the small intestine (Breves and Schroder, 1991). After a P rich meal, there are elevated levels of P in the small intestine. These elevated levels of P are primarily taken up by the passive non-Na-dependent pathway to avoid saturation and unwarranted energy use in active transport (Gueguen and Rerat, 1967). After the P crosses the brush-border membrane, it must travel within the enterocytes to the basal membrane. Once at the basal membrane, P diffuses into the blood stream (Anderson and Garner, 1996).

Metabolism of Absorbed P

Phosphorus can be transported in the blood in both the organic form as phospholipids, and as inorganic P that is attached to serum proteins (Pond et al., 2005). After P is incorporated into the blood serum it can either be utilized by the animal or stored with calcium in a 2:1 ratio as hydroxyapatite crystal in the bones (Anderson et al., 2006). Evaluating the metabolism of P requires a multifaceted approach due to the many functions of P in the body (Pond et al., 2005). Phosphorus metabolism includes P storage and mineralization from the bones, phospholipid metabolism, and metabolism of high energy bonds (ATP – ADP – AMP). The animal body is able to mobilize stored P from the bones in times of dietary deficiency. Mobilized P can be used for all body functions that require P.

Phospholipids represent a small portion of P in the body of the animal, but are important for cell structure as they form the lipid bilayers that give cells their shape. Phospolipids also serve as the phosphate backbone for glycerophospholipids. Phosphorus, therefore, indirectly

plays a key role in the metabolism and transport of lipids and cholesterol (Vance and Vance, 2008).

Phosphorus is a key element in many other biological and biochemical processes through its indirect role as an energy supplier. Adenosinetriphosphate is a high energy molecule synthesized in the mitochondrial membrane. In nearly all energy requiring processes, ATP is oxidized to release energy and create ADP, which can be further metabolized to AMP. The energy is released from the cleavage of the high energy bond between phosphates (Garrett and Grisham, 2002). The ATP molecule is vital for the survival of the animal and is involved in active transport across membranes, protein synthesis, and heat production in times of unfavorable thermoregulation by producing mechanical heat from muscles (Sherwood et al., 2008). Thus, ATP is a small but important use of P in swine.

Maintaining P Balance and Excretion

All metabolic functions and excretion of P are a result of endocrine regulation through parathyroid hormone (**PTH**) and its antagonist calcitonin (**CT**; Macintyre, 1989). The hormonal regulation is a feedback loop that integrates multiple organs and involves the interactions among hormones, stored Ca and P, vitamin D, and circulating P. The regulation of P shares common pathways with Ca regulation and homeostasis (Koeppen and Stanton, 2009). The feedback loop starts when the body responds to the total level of P in the body. In time of high P levels, the body goes into a state of hyperphosphatemia. The thyroid responds by secreting CT which down-regulates PTH (Jongbloed, 1987). In a hyperphosphatemic state, there is a lower urine phosphate level causing high serum phosphate levels, which in turn lower the concentration of calcitriol or 1,25-*dihydroxyvitamin D*3 (**1,25(OH)$_2$D$_3$**), a metabolic form of vitamin D. Lowered

levels of $1,25(OH)_2D_3$ will act in conjunction with CT to increase bone mineralization and decrease intestinal absorption. Meanwhile the lowered levels of PTH act on the proximal tubules of the kidneys to increase renal excretion of P (Koeppen and Stanton, 2009). This encompasses one half of the feedback loop needed to lower plasma levels of P. The complementary half is activated under conditions of hypophosphatemia, low P levels. If there are low P levels in the blood, the thyroid will increase secretion of PTH and increase intestinal absorption of P. High PTH and low CT will act on the kidneys causing reabsoption of P in the proximal tubules, thus increasing $1,25(OH)_2D_3$ levels and possibly also increasing intestinal absorption (Lei et al., 1994). Parathyroid hormone will also act on the bones and signal the osteocytes to release P from bone mass. This demineralization process will increase P in the blood, thus signaling increased CT and lowered PTH and completing the feedback loop. This process explains how the kidneys and the gut are the main routes for excretion of P in monogastric animals.

In the case of extreme P deficiency, rapid deterioration of the animal's condition may be observed. Deficiency can result in reduced appetite, lowered growth, and reduced feed conversion (Reinhart and Mahan, 1986). If P is deficient in the animal diet for a prolonged period of time, a medical condition known as rickets will develop (Pond et al., 2005). Other long-term effects of prolonged P deficiency are deformation of the bones and teeth due to demineralization (Miller et al., 1964). Deficiency symptoms may also be observed in cases with inadequate vitamin D or Ca in the diet as they are required for absorption and bone deposition (Koch and Mahan, 1985). Posterior paralysis, a condition of lameness in the hind legs, is common in high producing sows fed a P-deficient diet.

As with most minerals, P can be toxic although in a practical situation it is unlikely that the animal will consume enough P to cause problems. If problems were to arise, it is generally

due to interactions or combinations between P and other minerals (Underwood and Suttle, 1999). If P is fed at high levels it has a laxative effect. Additionally, high levels of P over a prolonged period of time can lead to renal failure due to the added stress of excretion (Pond et al., 2005).

Dietary Considerations

There are several key considerations in the calculation of P for diets including biochemical interactions and variations, effects of processing, and the addition of enzyme supplements and their effect on P utilization (NRC, 1998). Most importantly, this all must be tied together into one mixed ration that is easy to produce, transport, and feed (Reese et al., 2010).

Calcium-P Interactions

The most researched interaction among minerals is that between Ca and P. Calcium and P are closely related due to several common biologic pathways and their common storage form (Pond et al., 2005). When formulating diets, concentrations of P are often calculated as available P and sometimes, the Ca:P ratio is calculated as well (NRC, 1998). The ratio between Ca and P should be between 1.1:1 and 1.25:1 when feeding a grain-soybean meal based diet (NRC, 1998). If the ratio between Ca and total P is increased to a ratio of 1.5:1, animal performance is reduced (Hanni et al., 2005). If available or digestible P is used to calculate the diets, the ratio between Ca and available P should be between 2:1 and 3:1 (Jongbloed, 1987; Qian et al., 1996). Increasing the ratio of Ca:P may reduce the concentration of bone ash (Liu et al., 1998). A greater Ca:P ratio will lower P absorption, decrease bone mineralization, lower P blood serum levels, and decrease performance with a loss in ADG (Mahan, 1982). However, the Ca:P ratio is not as important for animal performance if P is excessively supplied in the diet (Hall et al.,

1991). When phytase is included in the diet, high dietary Ca concentrations can reduce the effectiveness of the phytase enzymatic activity, and thus reduce the availability of P (Qian et al., 1996). However, there is no effect on phytase function or P absorption when changing the ratio of Ca:P from 1.7:1 to 1.1:1 if 1,250 FTU of phytase is used (Cromwell et al., 1995). Thus, high Ca levels affect phytase only if phytase is fed at a low concentration (Hanni et al., 2005). There are 3 possible explanations for the negative effects of a wide Ca:P ratio in diets. High Ca levels decrease microbial and enzymatic activity by increasing the pH of the gut (Sandberg et al., 1993). Excess Ca may also render phytate bound P unavailable for hydrolization, and therefore reduce absorption by forming an insoluble phytate complex (Fisher, 1992). Phytase activity may also be reduced at high levels of Ca as Ca competes for activation sites on the enzyme leading to lowered phytase activity and reduced P digestibility (Qian et al., 1996). If feed intake is low, dietary concentrations of Ca and P may need to be increased to support animal performance (NRC, 1998).

Other Element Interactions

There are other interactions that may affect P availability and absorption. High levels of vitamin D can mobilize both Ca and P from the bones resulting in lowered bone density (Jongbloed,1987). Vitamin D is also required for absorption of P in the small intestine. In an outdoor production scheme, animals are supplied adequate vitamin D from the sun through the conversion of pro-vitamin D to activate vitamin D. In most present situations, animals are housed indoors where they have limited access to direct sunlight rendering this pathway inadequate. Therefore, vitamin D needs to be supplemented through the diet (Jongbloed, 1987). Most feedstuffs contain little or no vitamin D so it must be acquired through supplementation (Crenshaw, 2001). Vitamin D is activated by PTH and aids in the absorption of P and in the

mineralization of bone (Andersen et al., 2006). Adequate vitamin D is especially important in times of low P concentration as the absorption effect is greater during such times (Peterlik and Wasserman, 1978).

Microminerals may also play a role in the availibility of P (NRC, 1998). The presence of high levels of Fe, Al, and Mg may adversely affect the absorption of P by the pig. High levels of these minerals may form complexes with P resulting in reduced P-digestibility, which is why it is important to maintain the proper balance among minerals in the diet (NRC, 1998).

Form of P in Feeds

Phosphorus can be presented in many forms to the pigs (Pointillart et al., 1989). For the animal to adequately utilize P from plant sources, the P must be hydrolyzed by the enzyme phytase, which is the enzyme that releases P from phytate (Pallauf and Rimbach, 1997). Rumen microbes secrete active phytase in the rumen, which increases P-digestibility, whereas pigs and poultry do not (Raun et al., 1956). Therefore, phytase is required in the diet to release P from plant ingredients (Cromwell, 1992).

There are practical implications to the addition of phytase to swine diets. In cases where phytase is not added to the diets, there is a need to add additional inorganic P to meet the requirement of P. In the case of phytase addition, there is more digestible P in the diet, therefore, reducing the need to add inorganic P (NRC, 1998). With more P being digested, there is less P in the manure, resulting in a reduced chance of environmental impact. This may improve both the costs of swine diets and the public perception of animal agriculture.

Sources of P in Diets

The main ingredients in swine diets are cereals, oilseeds, and their by-products (Godoy et al., 2005). Among these ingredients, there are differences in the availability of P (NRC, 1998). These differences can be accounted for by looking at the form of P in the source. The concentration of P in cereal grains ranges from 0.25% to 0.45 %. Oilseeds have a slightly greater total P concentration ranging from 0.45 to 0.75% (Eeckhout and De Paepe, 1994). Of the total P in cereal grains and oilseeds, 60-85% is bound in phytate (Lolas et el., 1976; Raboy, 1997). Of the P in corn, as little as 15% (Trotter and Allee, 1979a,b) is digestible P compared with 50% in wheat (NRC, 1998). Wheat P is more bio-available due to the presence of endogenous phytase in wheat (Pointillart et al., 1984). In comparison, feed ingredients of animal origin have high digestibility of P ranging from 67 to 90% (Traylor et al., 2005).

Two of the most common sources of inorganic P used in swine diets are dicalcium phosphate (**DCP**) and monocalcium phosphate (**MCP**). Much like in cereal grains, there are also variations in the digestibility of P among inorganic sources of P (NRC, 1998). The digestibility of P in MCP and DCP is between 80 and 90 %, but monosodium phosphate (**MSP**) has a greater digestibility of P than MCP and DCP (Petersen and Stein, 2006).

Influence of BW and Age on P Digestibility

The use of microbial phytase has led to greater utilization of dietary P in swine and if proper methodology is used, the digestibility of P in feed ingredients can be accurately determined. However, there are potential factors other than the ingredients that play a role in the digestibility of P in swine because the physiological stage of the pig may have an impact on P requirement and utilization. Phosphorus is needed for lean deposition, gestation, and milk

production (NRC, 1998). There is an increased requirement in gestating animals for fetal development and during lactation depending on milk production levels (NRC, 1998). The effect of BW on the requirement of P is well documented, but the effect that BW has on P digestibility is unknown. Kemme et al. (1997) focused on factors affecting P digestibility in various housing systems. They compared the digestibility of P in pigs weighing 60, 75, and 90 kg in both metabolism cages and regular penning systems and concluded that BW had no effect on P-digestibility. But no data for a wider BW exist.

There is no difference in the requirement of P for animals with a high growth rate and those who grow at a slower pace (Bertram et al., 1994). However, if animals are treated with porcine somatotropin, they have a reduced feed intake, thus increasing the dietary requirement of P if it is expressed as a percentage of the diet (Weeden et al., 1993a,b). There is also a higher growth rate due to the use of somatotropin, which also increases the P requirement (Carter and Cromwell, 1998a,b).

Influence of Diet Preparation on P Digestibility

The method of diet preparation may affect the digestibility of P in diets fed to swine. Cold pelleting has no effect on P digestibility or phytase activity (Corley et al., 1980), but steam pelleting may decrease P digestibility. When the temperature rises to about 80 ^{o}C there is a significant decrease in P digestibility due to destruction of the phytase in the ingredients (Jongbloed and Kemme, 1990). Steeping of diets also has an impact on P digestibility (Skoglund et al., 1997). Grain is steeped during wet milling for starch production and in liquid feeding systems. Along with the growth advantages that liquid feeding offers, steeping the diet also activates natural enzymes in the cereal products (Reddy et al., 1982). In a study by Skoglund et

al. (1997) it was concluded that by steeping a barley-rapeseed-pea diet, there was a 45% reduction in phytate and a 3-fold increase in the free P. The reason for this change is that the intrinsic phytase is activated, which reduces the amount of phytate in the diet (Carlson and Poulsen, 2003). The activated enzymes and water reduce the inositol phosphate in soaked feed, which results in higher digestibility of P (Lyberg et al., 2005). Distillers dried grains with solubles from the ethanol industry have greater digestibility of P than the corn they are produced from (Stein and Shurson, 2009). This is due to the fermentative process that hydrolyzes the bonds in phytate (Shurson et. al., 2004).

Expression of P Availability and Digestibility.

The requirement of P for pigs is established for all stages of production (NRC, 1998). However, there is not an established method for determining the digestibility or availability of P in feed ingredients or diets.

The historic approach has been to calculate relative bioavailability (RBV) using a slope-ratio method (Cromwell, 1992). Using this method has proven useful for ranking P availability among ingredients, but the procedure does not allow for the determination of P digestibility. To calculate RBV values, a P source of unknown availability is compared with a standard source of P in which P is highly digestible. The test ingredient and the P standard are included at graded levels in diets fed to pigs with all treatments being below the animal's actual requirement. After the end of a 4 to 6 week test period, animals are harvested and bones are extracted. Bone breaking strength, total bone ash, or P in bone ash is then regressed on P-intake for each source. The equation to calculate the relative bio-availability is:

$$\text{relative bioavailability} = 100 \text{ (slope B/slope A)}$$

where slope B is the slope that was obtained for the regression of the test ingredient and slope A is the slope for the standard (Cromwell, 1992). With the RBV procedure, the availability of P in the standard P source is given an arbitrary value of 100% and the availability of P in the test ingredient is expressed relative to the standard. Values cannot be compared across studies if different standards are used, and values are not additive in mixed diets. Additionally, the procedure is both labor intensive and costly.

An alternate method for estimating the available P in a diet or ingredient is to measure the digestibility of P. Digestibility is measured by calculating the differences between P-intake and fecal P excretion (Jongbloed et al., 1991). There are 2 common methods to determine digestibility: the direct method and the difference method. Using the difference method, a P source is added to a basal diet that contains a basal level of P. The difference in the digestibility of P in the basal diet and the basal diet containing the test P source is then calculated and from this value, the digestibility of P in the test source is calculated. If the direct method is used, a P source is included in a diet where all other ingredients are P-free. The digestibility of the P source is calculated by calculating the digestibility of P in the diet (Adeola, 2001). Both the difference and direct methods can be used to determine the apparent total tract digestibility (**ATTD**) of P in feed ingredients. Values for ATTD are calculated by using the following equation:

$$ATTD(\%)= [(Pi-Pf)/Pi]\times100$$

where Pi is P intake and Pf is P output in the feces (Almeida and Stein, 2010). The main drawback of using the ATTD method is that the endogenous losses of P are not accounted for,

which may lead to artificially reduced values for the ATTD of P in diets or ingredients with a low concentration of P.

Endogenous losses of P occur from the animal body at all times. These endogenous losses account for significant fecal P output that is not from the diet (Fan et al., 2001) and are derived from the natural turnover of bodily cells and secretions because most endogenous P originates from sloughed cells, spent enzymes, and the mucus lining of the cells (Garrett and Grisham, 2002). By taking endogenous losses into account, the animal's actual P needs are better determined (Dilger and Adeola, 2006). New techniques for measuring endogenous losses of P were proposed by Fan et al. (2001) who used the regression procedure, and by Petersen and Stein (2006) who used a P-free diet to determine endogenous losses of P. The regression procedure will result in values for total endogenous losses being determined, whereas the P-free diet will result in determination of basal endogenous losses of P. If values for ATTD of P are corrected for total endogenous losses, values for the true digestibility of P is calculated, but if ATTD values are corrected for basal endogenous losses, values for standardized total tract digestibility of P (**STTD**) are calculated.

Using the STTD method is believed to be a more accurate way of calculating the digestibility of P in ingredients and diets. Values for STTD of P are calculated using the following equation (Almeida and Stein, 2010):

$$\text{STTD } (\%) = [\text{P}i - (\text{P}f - \text{EPL})/\text{P}i] \times 100$$

where EPL is endogenous P losses. If STTD is used to determine the digestibility of P in ingredients it is believed that values that are additive in mixed diets are derived. Experiments to

measure STTD values of P in feed ingredients are less costly than experiments to measure RBV values as there is no need to harvest animals at the end of the experiment.

Conclusions

There is a need for further research into factors affecting the STTD and endogenous P losses. Future research will focus on validating the STTD methods using P-free diets by comparing them to RVB data obtained by using the same ingredients. Furthermore, there is a need to further examine the endogenous losses of P from animals kept under different conditions. Using STTD may lead to more accurate digestibility values and digestibility values obtained using STTD methods may be compared among experiments. In addition, STTD values are believed to be additive in mixed diets.

This thesis will focus on the STTD method for determining digestibility of P in feed ingredients. Specifically, the objectives of this thesis are to:

1. Compare values for STTD and relative bioavailability of P in distillers dried grains with solubles.

2. Evaluate the effects of pig BW on endogenous losses and the STTD values of feed ingredients.

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CHAPTER 3

COMPARISON OF VALUES FOR STANDARDIZED TOTAL TRACT DIGESTIBILITY AND RELATIVE BIOAVAILABILITY OF PHOSPHORUS IN DICALCIUM PHOSPHATE AND DISTILLERS DRIED GRAINS WITH SOLUBLES FED TO GROWING PIGS

ABSTRACT: Two experiments were conducted to compare values for the standardized total tract digestibility (STTD) and the relative bioavailability of P in dicalcium phosphate (DCP) and distillers dried grains with solubles (DDGS) when fed to growing pigs. In Exp. 1, the apparent total tract digestibility (ATTD), the basal endogenous P loss (EPL), and the STTD of P in DCP and DDGS were determined. Eighteen pigs (initial BW: 34.93 ± 1.04 kg) were allotted to 3 cornstarch-based diets in a randomized complete block design and housed individually in metabolism cages. Two diets contained DCP and DDGS, respectively, as the sole source of P and the last diet was a P-free diet that was used to measure EPL from the pigs. Results indicated that the ATTD of P in DCP and DDGS were 86.1 and 58.8%, respectively, and the STTD of P in DCP and DDGS were 93.1 and 63.1%, respectively. The EPL was determined at 174 mg/kg DMI. In Exp. 2, forty two pigs (initial BW: 29.02 ± 2.03 kg) were allotted to 7 dietary treatments in a randomized complete block design. Pigs were housed individually and allowed ad libitum access to feed and water. A basal diet (0.22% P) based on corn, casein, cornstarch, and potato protein concentrate was formulated. Three additional diets were formulated by adding 0.04, 0.08,

or 0.12% P from DCP to the basal diet to create diets containing 0.26, 0.30, or 0.34% P. The last 3 diets were formulated by adding 0.04, 0.08, or 0.12% P from DDGS to the basal diet at the expense of cornstarch. Pigs were fed experimental diets for 28 d. They were then euthanized and the third and fourth metacarpals from the right front foot were collected. Metacarpal bone ash and bone P were regressed against P intake for each ingredient and via slope ratio methodology, it was determined that the bioavailability of P in DDGS was 87% relative to that in DCP. It was concluded from this work that there is not good agreement between values for the STTD of P and the relative bio-availability of P in DDGS.

Key words: bioavailability, dicalcium phosphate, distillers dried grains with solubles, phosphorus, pigs, standardized digestibility

INTRODUCTION

Traditionally, P availability of feed ingredients has been determined by measuring the relative bioavailability (**RBV**) of P using a slope ratio method (Cromwell, 1992). This method works well if the objective is to compare and rank different sources of P. However, the RBV procedure does not allow for calculation of the digestibility of P in a specific ingredient. Calculating the RBV of P also does not allow for calculation of the quantities of P absorbed and excreted by the pig. If the total tract digestibility of P in individual feed ingredients is determined, the amount of P absorbed by the pig as well as the amount of P excreted in the feces from the pig can be calculated (Petersen and Stein, 2006).

Total tract digestibility of P may be measured as apparent total tract digestibility (**ATTD**) or standardized total tract digestibility (**STTD**). Values for ATTD and STTD may be measured

directly in P-containing ingredients and values are not expressed relative to values for a standard or a control diet. Endogenous losses of P (**EPL**) represent a small, yet significant, source of P excretion that needs to be accounted for when calculating digestibility values (Fan et al., 2001). Values for STTD of P are calculated by correcting values for ATTD of P by basal endogenous losses and unlike values for ATTD of P, values for STTD of P are believed to be additive in mixed diets (Almeida and Stein, 2010). Basal EPL may be measured using a P-free diet (Petersen and Stein, 2006). To our knowledge there are, however, no reports on the comparison of values for STTD of P and the RBV of P. Therefore, the objective of this experiment was to measure the STTD of P in distillers dried grains with solubles (**DDGS**) and to compare this value to the bioavailability of P in DDGS relative to the bioavailability of P in dicalcium phosphate (**DCP**).

MATERIALS AND METHODS

Both animal protocols concerning this work were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois.

Digestibility of P in DCP and DDGS, Exp. 1

Pigs, Diets, Experimental Design, and Housing. Commercial sources of DCP (PCS Sales LLC., Northbrook, IL) and corn DDGS (Lincolnland Agri Energy LLC, Palestine, IL) were obtained (Table 3.1). Two diets containing DCP or DDGS as the sole source of P were formulated (Table 3.2). A P-free diet that was used to measure basal endogenous losses of P was also formulated.

A total of 18 growing barrows (Genetiporc, Alexandria, MN) with an initial BW of 34.93 ± 1.04 kg were randomly allotted to the 3 diets with 6 replicates per diet using a randomized complete block design. Pigs were placed in metabolism cages that were equipped with a feeder and a nipple drinker, and had expanded metal floors. Screens were placed under the floors, which allowed for total collection of feces.

Feeding and Sample Collection. Animals were fed their respective diets for 12 d at a level of 2.5 times the daily maintenance requirement for energy (i.e., 106 kcal ME per kg $BW^{0.75}$; NRC, 1998). The daily quantity of feed was provided in 2 equal meals at 0500 and 1700. Water was available at all times. The initial 5 d adaptation period to the diets was followed by a 5 d collection period. A marker was added to the morning meals on d 6 and 11 and feces were collected using the marker to marker approach (Adeola, 2001). Chromic oxide was used at the beginning of collection and indigo carmine was used to mark the end of collection. Fecal samples were stored at -20°C immediately after collection.

Data Recording and Chemical Analysis. Initial and final BW of the pigs were recorded along with feed intake, feed refusals, and passage of the markers. At the conclusion of the experiment, fecal samples were removed from storage, dried in a forced air oven and finely ground using a model 4 Thomas-Wiley laboratory mill with a 1.0 mm sieve (Thomas Scientific, Philadelphia, PA). Fecal samples and diets were analyzed for DM by forced air oven drying at 135°C for 2 h (method 930.15; AOAC International, 2007). Fecal samples, diets, DCP, and DDGS were also analyzed for P and Ca by inductively coupled plasma spectroscopy (method 985.01; AOAC International, 2007) after wet ashing (method 975.03; AOAC International, 2007). The DDGS sample was analyzed for CP (method 990.03, AOAC International, 2007) and

AA (method 982.30 E (a, b, c); AOAC International, 2007), and diets and the DDGS sample were analyzed for ADF (method 973.18; AOAC International, 2007), and NDF (Holst, 1973).

Calculations and Statistical Analyses. Values for ATTD, EPL, and STTD were calculated as described by Almeida and Stein (2010). Data were analyzed by ANOVA using the Proc GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The main effect was treatment and treatment means were calculated using the LSMeans procedure. The pig was the experimental unit and an alpha value of 0.05 was used to asses significance between means. Homogeneity of the variances between treatments was confirmed and outliers were identified using the UNIVARIATE procedure as values that had treatment means of more than 3 times the interquartile range (Devore and Peck, 1993).

Bioavailability of P, Exp. 2

Pigs, Diets, Experimental Design, and Housing. Forty-two barrows (Genetiporc, Alexandria, MN) weighing 29.02 ± 2.03 kg were allotted to 7 dietary treatments with 6 replicate pigs per diet in a randomized complete block design using the Experimental Animal Allotment Program (Kim and Lindemann, 2007). Pigs were housed individually in pens (0.9 x1.8 m) that had a fully slatted concrete floor and a feeder and a nipple drinker.

A corn-based basal diet containing 0.22% P was formulated (Table 3.3). This diet contained potato protein concentrate (Avebe U.A., Veendam, The Netherlands), and casein (International Ingredients Company, Saint Louis, MO) as protein sources. Three additional diets were formulated by adding 0.04, 0.08, or 0.12% P from DCP to the basal diet resulting in a diet containing 0.26. 0.30, or 0.34% P. The last 3 diets were formulated by adding 0.04, 0.08, or

0.12% P from DDGS to the basal diet at the expense of cornstarch. The DCP and DDGS that were used in this experiment were from the same batches as those used in Exp. 1.

Feeding and Sample Collection. Individual pig BW were recorded at the beginning and at the conclusion of the experiment. Animals were allowed ad libitum access to feed and water throughout the 28-d experiment. Daily feed allowances and feed refusals were recorded. Subsamples of all diets were collected and stored at -20°C in zip lock freezer bags until analyzed. Feed refusals were stored at -20°C as well.

At the conclusion of the experiment, all animals were euthanized via captive bolt stunning and the right front foot was removed at the hock joint. The third and fourth metacarpals of the right foot were removed and cleaned to remove soft tissue. Bones were then sealed in plastic freezer bags and stored at -20°C. The metacarpals were later removed from storage and thawed, and bones were broken to expose the marrow and soaked in petroleum ether to remove fat and marrow. Bones were then placed under a chemical hood and air dried for 24 h and dried overnight at 130°C in an Isotemp 500 series gravity convection oven (Fisher Scientific, Pittsburg, PA) prior to ashing in an Isotemp muffle furnace (Fisher Scientific, Pittsburg, PA) for 6 h at 600°C (method 975.03; AOAC International, 2007). The weight of the ash was recorded.

***Chemical Analysis*.** Procedures for chemical analyses were identical to those used in Exp. 1. Diets were analyzed for DM, CP, GE, ash, ADF, and NDF (Table 3.5). Diets and all ashed bones were also analyzed for P and Ca.

Calculations and Statistical Analyses

Data were analyzed as a complete randomized block design using the MIXED procedure (SAS Inst., Cary, NC) with pig as the experimental unit. Outliers were determined using the

same procedure as described for Exp. 1. Orthogonal polynomial contrasts were performed to test linear and quadratic responses to increasing levels of P intake. Relative bioavailability of P in DDGS was determined by means of multiple linear regressions and the slope-ratio method using DCP as the standard source. Bone ash weight and bone P content were regressed on P intake using the basal diet as a common intercept as described by Littell et al. (1997). Regression using bone ash weight and bone P data met all 3 assumptions for the slope-ratio assay procedure, that the responces are linear, the lines share a common intercept and that the reponce aat the zero level is equal to the common intercepts. The RBV of P in DDGS was then calculated by dividing the slope of the regression line for DDGS by the slope of the regression line for DCP (Cromwell, 1992). An α-level of 0.05 was used in all data analyses and $0.05 < P < 0.10$ was used to indicate tendency.

RESULTS

Digestibility of P in DCP and DDGS, Exp. 1

All pigs easily consumed their respective diets and remained healthy throughout the experiment. However, 1 pig on the DCP treatment was identified as an outlier and was removed from the data because data for ADFI, ATTD of P, and STTD of P for this pig were greater than 3 times the interquartile range. Pigs fed the DCP diet consumed more ($P < 0.05$) feed than pigs fed the DDGS diet (Table 3.6). Daily intake of P and the fecal output of P were greater ($P < 0.001$) for pigs fed the DDGS diet than for pigs fed the DCP diet. The ATTD and STTD of P were, however, greater for DCP than for DDGS ($P < 0.001$).

Bioavailability of P, Exp. 2

One pig that was fed the basal diet died during the experiment and was not included in the statistical analysis. One pig consuming the DCP diet with 0.30% P and one on the DCP diet with 0.34 % P were identified as outliers and were also excluded from calculations.

All animals gained weight during the experiment. Final BW, ADG, ADFI, and G:F increased (linear, $P < 0.05$) as the concentration of P in the diet from DDGS increased (Table 3.7). There was also a tendency ($P < 0.10$) for a linear increase in the final BW and there was an increase ($P < 0.05$) in ADFI and ADG as increasing levels of P from DCP was included in the diet.

The dry fat free bone weight increased (linear, $P < 0.05$) as DCP was added to the basal diet and there was no effect in bone weight as increasing concentrations of DDGS were included in the diet (Table 3.8). The amount of bone ash increased as dietary P level increased, regardless of the source of P (linear, $P < 0.01$). The concentration of P and Ca if measured as a percent of bone was not influenced by dietary treatments. However, the total amount of P and Ca increased (linear $P < 0.01$) as the level of P in the diet increased, regardless of the source of P. The bioavailability of P in DDGS relative to the bioavailability of P in DCP was 86% if calculated from total bone ash weight and 88% if calculated from the amount of P in the bone ash (Table 3.9, Figure. 3.1, Figure. 3.2).

DISCUSSION

Inorganic P sources such as DCP are added to diets as P supplements and DCP may also be used as the standard when the RBV of other ingredients is determined (Cromwell, 1992). The

ATTD of P in DCP that was calculated in this experiment is in agreement with values reported by Rodehutscord et al. (1994) and Petersen and Stein (2006), but greater than the value of 73% reported by Eeckhout and de Paepe (1997). However, differences in the ATTD of P among different sources of DCP have been reported (Jongbloed, 1987).

The value for the basal EPL (174 g/kg DMI) that was measured for pigs fed the P-free diet is within the range of reported values between 139 and 211 mg/kg of DMI (Petersen and Stein, 2006; Stein et al., 2006; Almeida and Stein, 2010). Correction of the ATTD values for basal EPL allows for calculation of STTD values for P, which are believed to be additive in mixed diets and, therefore, more relevant for practical feed formulation than values for ATTD (Almeida and Stein, 2010). The STTD of P in DCP (93.12%) concurs with the value determined by Petersen and Stein (2006) in a different source of DCP. This value indicates that the source of DCP that was used in this experiment was of high quality because it had excellent digestibility of P.

The ATTD of P in DDGS (58.8%) is in agreement with previous values that range from 50 to 69% (Pedersen et al., 2007; Stein et al., 2009; Almeida and Stein, 2010) and is very close to the average ATTD of P in DDGS, which is 59% (Stein and Shurson, 2009). The STTD of P in DDGS was less than the value of 72.9% reported by Almeida and Stein (2010), but it is recognized that some differences in the digestibility of P among sources of DDGS exist (Stein and Shurson, 2009). Variability in the digestibility of P among sources of DDGS may be attributed to differences in production systems among ethanol plants (Pedersen et al., 2007).

The fact that pigs fed the basal diet in Exp. 2 had the least bone ash and that ash weight increased with P concentration in the diets was expected and is in agreement with Cromwell et

al. (1970) and Traylor et al. (2005). The total quantity of P in bone ash is an accurate measure of P deposition in bones because P is stored in a mineral form, which is analyzed as bone ash. As more P is absorbed, more bone is synthesized, and the quantity of bone ash increases. Phosphorus is deposited in bone ash at a relatively constant concentration, which is the reason there was no differences in the percent of P in bone ash among treatments. This observation indicates that the regulation of P deposition is at the level of total bone ash synthesis whereas the composition of the bone ash does not change. This conclusion is in agreement with data reported by Petersen et al. (2011) and indicates that both total bone ash and total bone P are accurate measures of P deposition, whereas P as a percent of bone ash does not change with the P-status of the pig.

The bioavailability of P in DDGS relative to P in monosodium phosphate has been reported at 77 and 84% (Fent et al., 2004; Jenkin et al., 2007). The RBV of P in DDGS that was determined in this experiment is slightle greater than these values and also greater than the value of 70% suggested by Burnell et al. (1989), who also used DCP as the standard as we did in this experiment. The value for the RBV of P in DDGS obtained in the present experiment was similar to the value of 89% reported by Whitney and Shurson (2001) and greater than the NRC value of 77% (NRC, 1998).

The value for the RBV in a feed ingredient can be calculated from the STTD value by dividing the STTD of P in the test ingredient by the STTD of P in the standard. In the present experiment, the STTD of P in DDGS was 63% and the STTD of P in DCP was 93%, which results in an estimated RBV of P in DDGS of 67%. This value is less than the determined value for RBV of P in DDGS.

Use of STTD values for P is assumed to allow for more accurate formulation of mixed diets than if values for RBV of P or ATTD of P are used because STTD values are believed to be additive in mixed diets, which is not always the case for values for ATTD and RBV because of the effect of the diet. The present data indicating that there is not good agreement between data for the RBV of P calculated from the STTD values and directly measured RBV-values further support the use of STTD values in practical feed formulation. To our knowledge, this is the first time such a comparison has been made.

CONCLUSION.

Results of the present experiment indicate that there is not a good agreement between data for RBV and STTD of P if the digestibility of P in the standard P-source is known These results, therefore, give support to the use of STTD values in feed formulation, because STTD values can be determined without relying on a standard, and values are believed to be additive in mixed diets.

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Table 3.1. Analyzed composition of dicalcium phosphate and distillers dried grains with solubles (DDGS) as-fed basis, Exp. 1 and 2[1]

	Ingredient	
Item	Dicalcium phosphate	DDGS
GE, cal/g	-	4,804
CP, %	-	28.18
DM, %	96.89	89.37
Ash, %	82.54	4.31
P, %	19.77	0.89
Ca, %	20.88	0.02
NDF, %	-	34.35
ADF, %	-	9.87

[1]Amino acids (%) were analyzed in DDGS as follows: Arg, 1.31; His, 0.76; Ile, 1.06; Leu, 3.18; Lys, 0.93; Met, 0.55; Phe, 1.23; Thr, 0.98; Trp, 0.19; Val, 1.43; Ala, 1.92; Asp, 1.74; Cys, 0.62; Glu, 3.75; Gly, 1.09; Pro, 2.10; Ser, 1.08; and Tyr, 0.90.

Table 3.2. Ingredient composition of experimental diets (as-fed basis), Exp. 1

	Diet		
Ingredient	P-free	Dicalcium phosphate	DDGS[1]
Dicalcium phosphate	-	1.25	-
DDGS[1]	-	-	40.00
Cornstarch	49.22	47.97	43.10
Sucrose	20.00	20.00	15.00
Soybean oil	4.00	4.00	-
Solka floc[2]	4.00	4.00	-
Ground limestone	0.80	0.80	1.20
Gelatin[3]	20.00	20.00	-
DL-Met	0.27	0.27	-
L-Thr	0.08	0.08	-
L-Trp	0.14	0.14	-
L-His	0.08	0.08	-
L-Ile	0.16	0.16	-
L-Val	0.05	0.05	-
Salt	0.40	0.40	0.40
Vitamin and mineral premix[4]	0.30	0.30	0.30
Potassium carbonate	0.40	0.40	-
Magnesium oxide	0.10	0.10	-

Table 3.2 (cont.)

[1]DDGS = distillers dried grains with solubles.

[2]Fiber Sales and Development Corp. (Urbana, OH).

[3]Gelita Gelatine USA Inc. (Sioux City, IA).

[4]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,128 IU; vitamin D3 as cholecalciferol, 2,204 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrocloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide and nicotinic acid, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Table 3.3. Ingredient composition of experimental diets (as-fed basis), Exp. 2

	Diets						
	Basal	Dicalcium phosphate			Distillers dried grains with solubles		
Ingredient % P:	0.22	0.26	0.30	0.34	0.26	0.30	0.34
Ground corn	64.95	64.81	64.81	64.81	64.81	64.81	64.81
Potato protein concentrate[1]	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Casein[2]	2.40	2.40	2.40	2.40	2.40	2.40	2.40
Cornstarch	22.00	22.05	21.97	21.88	17.52	12.90	8.24
Soybean oil	1.80	1.80	1.80	1.80	1.38	0.95	0.52
Dicalcium phosphate	-	0.22	0.44	0.66	-	-	-
Distillers dried grains with solubles	-	-	-	-	5.20	10.40	15.60
Ground limestone	1.42	1.29	1.15	1.02	1.41	1.40	1.38
L-Lys-HCL	0.40	0.40	0.40	0.40	0.35	0.31	0.26
DL-Met	0.12	0.12	0.12	0.12	0.06	0.01	-
L-Thr	0.10	0.10	0.10	0.10	0.07	0.03	-

Table 3.3 (cont.)

L-Trp	0.06	0.06	0.06	0.06	0.05	0.04	0.04
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin and mineral premix [3]	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Tylan premix [4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05

[1]Avebe U. A. (Veendam, The Netherlands).

[2]International Ingredients Company (Saint Louis, MO).

[3]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,128 IU; vitamin D3 as cholecalciferol, 2,204 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrocloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide and nicotinic acid, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Table 3.3 (cont.)

[4]The Tylan premix (Elanco Animal Health, Greenfield, IN) included at 0.05% provided 44 mg tylosin in the form of tylosin phosphate per kilogram of complete diet.

Table 3.4. Analyzed composition of experimental diets (as-fed basis), Exp. 1

	Diet		
Item	P-free	Dicalcium phosphate	Distillers dried grains with solubles
ME, kcal/kg[1]	3,452	3,403	3,660
DM, %	92.62	92.72	90.96
Ash, %	1.25	2.21	3.02
P, %	-	0.23	0.37
Ca, %	0.36	0.57	0.59
NDF, %	4.03	6.79	16.73
ADF, %	2.96	1.72	5.08

[1]Values for ME were calculated (NRC, 1998) rather than analyzed.

Table 3.5. Analyzed composition of experimental diets (as-fed basis), Exp. 2

		Diet						
		Basal diet	Dicalcium phosphate			Distillers dried grains with solubles		
Ingredient	% P:	0.22	0.26	0.30	0.34	0.26	0.30	0.34
ME, mcal/kg[1]		3,567	3,564	3,561	3,557	3,526	3,484	3,440
DM, %		88.42	88.50	88.85	89.24	89.02	88.44	88.17
Ash, %		2.58	2.62	2.71	2.95	2.81	2.93	3.51
CP, %		11.24	11.38	11.49	11.25	13.11	13.96	15.31
P, %		0.24	0.23	0.28	0.35	0.23	0.28	0.32
Ca, %		0.52	0.59	0.66	0.62	0.60	0.63	0.64
NDF, %		8.22	6.37	6.40	8.08	7.66	10.88	11.85
ADF, %		1.92	1.56	1.54	2.10	2.38	3.33	3.83

[1]Values for ME were calculated (NRC, 1998) rather than analyzed.

Table 3.6. Intake, output, and digestibility of P in dicalcium phosphate (DCP) and distillers dried grains with solubles (DDGS) fed to growing pigs, Exp. 1

	Diet			
Item	DCP	DDGS	SEM	*P*-value
Feed intake, g/d	1,023	925	20	0.005
P intake, g/d	2.5	3.8	0.2	< 0.001
Fecal P output, g/d	0.3	1.6	0.2	< 0.001
ATTD, %[1]	86.1	58.8	3.8	< 0.001
STTD, %[2]	93.1	63.1	3.8	< 0.001

[1]ATTD = apparent total tract digestibility.

[2]STTD = standardized total tract digestibility. Values for STTD were calculated by correcting ATTD values for basal endogenous losses. Basal endogenous losses of P were determined to be 174 mg/kg DMI.

Table 3.7. Effects of dietary P from dicalcium phosphate (DCP) or distillers dried grains with solubles (DDGS) on pig growth performance, Exp. 2

	Diet and P source							DCP			DDGS		
	Basal	DCP			DDGS			*P*-value			*P*-value		
Item % P:	0.22	0.26	0.30	0.34	0.26	0.30	0.34	SEM	L[1]	Q[1]	SEM	L[1]	Q[1]
Initial wt, kg	29.82	29.03	29.48	29.78	29.03	29.10	28.60	0.84	0.93	0.55	0.84	0.37	0.87
Final wt, kg	46.94	48.82	49.94	52.26	45.50	51.55	52.96	2.00	0.08	0.92	2.00	0.01	0.49
ADG, kg	0.61	0.71	0.73	0.81	0.59	0.80	0.87	0.06	0.04	0.85	0.06	< 0.01	0.45
ADFI, kg	1.70	1.89	2.11	2.15	1.65	2.01	2.06	0.09	< 0.01	0.47	0.09	< 0.01	0.62
G:F	0.35	0.37	0.35	0.37	0.36	0.40	0.42	0.02	0.71	0.90	0.02	< 0.01	0.58

[1] L = linear effect; Q = quadratic effect.

Table 3.8. Effects of dietary P from dicalcium phosphate (DCP) or distillers dried grains with solubles (DDGS) on bone traits, Exp. 2

	Diet and P source							DCP			DDGS		
	Basal	DCP			DDGS			*P*-value			*P*-value		
Item % P:	0.22	0.26	0.30	0.34	0.26	0.30	0.34	SEM	L[1]	Q[1]	SEM	L[1]	Q[1]
Bone wt, g[2]	6.98	6.94	7.75	8.29	6.42	6.93	7.48	0.45	0.04	0.55	0.45	0.34	0.24
Bone ash wt, g	2.61	2.63	3.12	3.38	2.63	2.87	3.16	0.10	< 0.01	0.25	0.10	< 0.01	0.21
Bone ash P, %	19.90	19.95	20.09	19.79	19.66	20.21	19.86	0.25	0.89	0.50	0.25	0.71	0.81
Bone P, g	0.52	0.52	0.63	0.67	0.52	0.58	0.63	0.61	< 0.01	0.42	0.61	< 0.01	0.32
Bone Ca, %	40.75	40.60	40.30	40.58	41.23	40.27	40.04	0.49	0.74	0.68	0.49	0.17	0.47
Bone Ca, g	1.06	1.07	1.26	1.37	1.08	1.16	1.26	0.04	< 0.01	0.17	0.04	< 0.01	0.29

[1] L = linear effect; Q = quadratic effect.

[2]Bone dry fat free weight.

Table 3.9. Linear regression and relative bioavailability (RBV) of P, Exp. 2

Parameter	Source	Slope	Intercept	*P*-value	RBV
Bone ash wt, g x P intake, g	DCP	3.32	2.44	< 0.01	100
	DDGS	2.85	2.44	0.03	86
Bone P, g x P intake, g	DCP	0.67	0.48	< 0.01	100
	DDGS	0.59	0.48	0.04	88

[1]DCP = dicalcium phosphate; DDGS = distillers dried grains with solubles.

Figure Legends

Figure 3.1. Linear regression of bone ash on supplemental P intake and relative bioavailability of P. DCP = dicalcium phosphate; DDGS = distillers dried grains with solubles. Values on the X-axis are based on analyzed values for P in the diets.

Figure 3.2. Linear regression of bone P on supplemental P intake and relative bioavailability of P. DCP = dicalcium phosphate; DDGS = distillers dried grains with solubles. Values on the X-axis are based on analyzed values for P in the diets.

Figure 3.1.

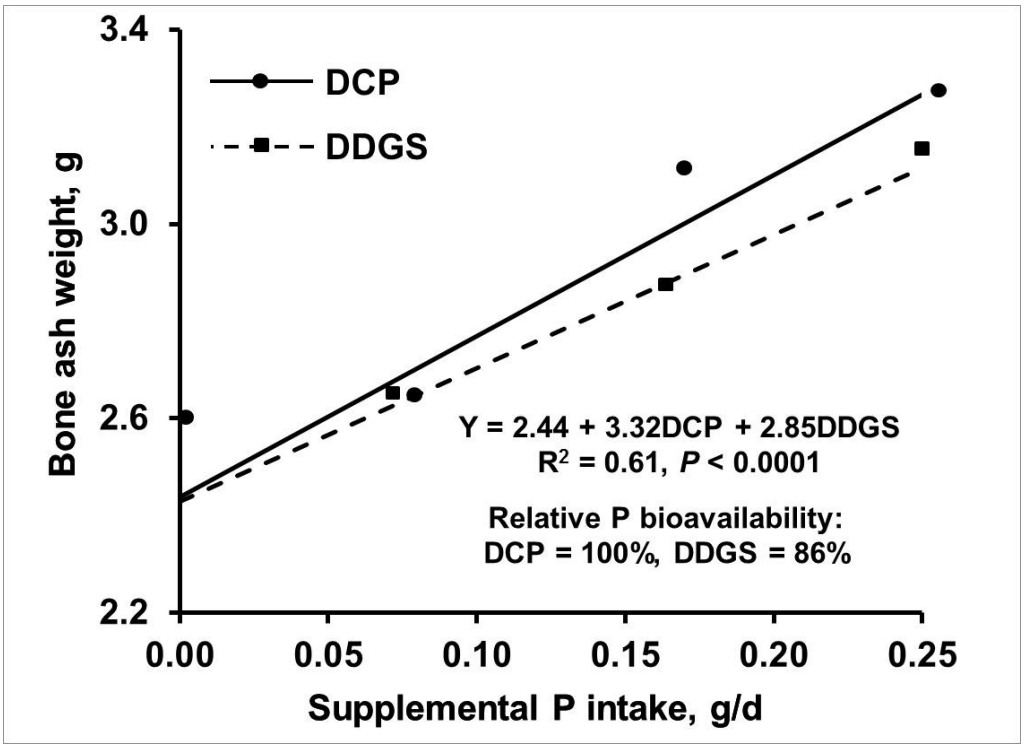
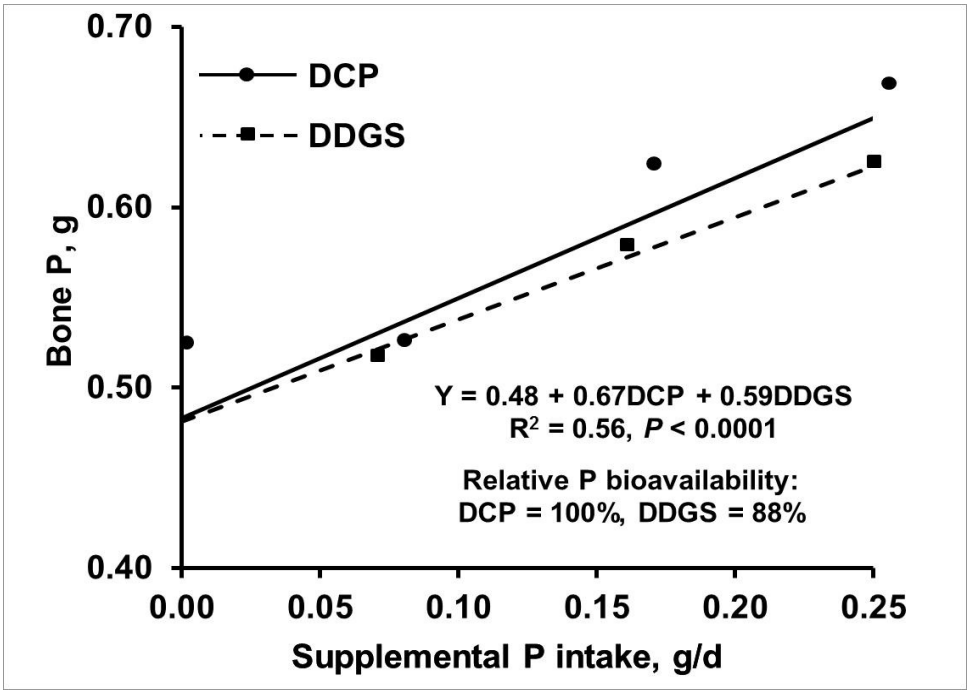

3.4
3.0
2.6
2.2
Bone ash weight, g
DCP
DDGS
Y = 2.44 + 3.32DCP + 2.85DDGS
R2 = 0.61, P < 0.0001
Relative P bioavailability:
DCP = 100%, DDGS = 86%
0.00
0.05
0.10
0.15
0.20
0.25
Supplemental P intake, g/d

Figure 3.2


0.70
0.60
0.50
0.40
Bone P, g
DCP
DDGS
Y = 0.48 + 0.67DCP + 0.59DDGS
R2 = 0.56, P < 0.0001
Relative P bioavailability:
DCP = 100%, DDGS = 88%
0.00
0.05
0.10
0.15
0.20
0.25
Supplemental P intake, g/d

CHAPTER 4

EFFECT OF BODY WEIGHT ON BASAL ENDOGENOUS LOSSES OF PHOSPHORUS AND TOTAL TRACT DIGESTIBILITY OF NUTRIENTS AND ENERGY BY GROWING PIGS

ABSTRACT: An experiment was conducted to determine the effect of pig BW on the apparent total tract digestibility (ATTD) of P, Ca, GE, CP, ether extract, ADF, NDF, and ash, on basal endogenous P losses (EPL), and on the standardized total tract digestibility (STTD) of P in soybean meal (SBM), distillers dried grains with solubles (DDGS), and in yellow dent corn. Three semi-purified diets in which SBM, DDGS, or corn was the sole source of P and a P-free diet that was used to determine EPL were formulated. Twenty-four barrows with an initial BW of 9.66 ± 0.67 were allotted to the 4 diets and placed in metabolism cages during five 12-d collection periods when they had an average initial BW of 9.66 ± 0.67, 22.29 ± 2.57, 53.77 ± 9.91, 92.73 ± 16.17, and 129.23 ± 18.55 kg. Pigs were fed their allotted diet throughout all 5 periods, but corn-SBM-DDGS diets were provided between collection periods. During collection periods, feces were collected from d 6 to 11 and values for ATTD, EPL, and STTD of P and ATTD of Ca, GE, CP, ether extract, ADF, NDF, and ash were calculated. Data were analyzed using orthogonal contrasts and repeated measures analysis. There was no effect of BW on EPL and the average EPL was 220 mg/kg DMI. The ATTD and STTD of P in SBM and corn increased (linear, $P < 0.01$) as the BW of pigs increased, but that was not the case for DDGS. For the SBM diets, there was an increase in the ATTD of CP (linear and quadratic, $P < 0.05$),

ether extract, GE, and ash (quadratic, $P < 0.05$) as pig BW increased, but there was no effect ($P > 0.05$) of BW on the ATTD of ADF, NDF, and Ca. For the DDGS diet, there was an increase in the ATTD of GE, NDF, and CP (linear and quadratic, $P < 0.05$), ether extract (linear, $P < 0.05$), and ADF (linear, $P < 0.05$), and of ash (quadratic, $P < 0.01$ as BW increased, but the ATTD of Ca in DDGS decreased (linear, $P < 0.01$) as pig BW increased. For corn, there was an increase (linear and quadratic, $P < 0.01$) in DE and in the ATTD of CP, ADF, and NDF (linear, $P < 0.05$) and of ash (linear and quadratic, $P < 0.01$) as pig BW increased, but there was no effect ($P > 0.05$) of pig BW on the ATTD of GE, ether extract, and Ca. It is concluded that the ATTD and STTD of P in corn and SBM, but not in DDGS, increases as the pig BW increases, but EPL of P is constant regardless of pig BW. The influence of pig BW on the ATTD of GE and nutrients is dependent on the diet.

Key words: apparent digestibility, body weight, endogenous losses, energy, phosphorus, pig

INTRODUCTION

Digestibility values for P can be measured as apparent total tract digestibility (**ATTD**) or standardized total tract digestibility (**STTD**) values. To calculate STTD values, ATTD values are corrected for basal endogenous losses of P (**EPL**) that may be measured using a P-free diet (Almeida and Stein, 2010). Values for basal EPL have been reported in the range of 139 to 211 mg/kg DMI (Petersen and Stein, 2006; Stein et al., 2006 a; Widmer et al., 2007; Almeida and Stein, 2010). All of these values were measured in pigs that had a BW between 10 and 78 kg and no values have been reported for the basal EPL of heavier pigs. Likewise, there are no values for the STTD of P in feed ingredients measured in pigs with a BW above 78 kg. The

ATTD of P in pigs with a BW of 60, 75, and 90 kg has been measured and within this range, there was no effect of BW on the ATTD of P (Kemme et al., 1997; Rodehutscord et al., 1999). However, to our knowledge, effects of BW on the ATTD of pigs over a greater BW range has not been measured, and it is not known if values for ATTD and STTD of P measured at a specific BW can be applied to pigs that have a different BW. Pig BW may affect energy digestibility of feed ingredients (Noblet et al., 1994; Noblet and van Milgen, 2004), and interactions between BW and diet composition should be taken into account when diets are formulated (Noblet et al., 1994). However, to our knowledge, there are no data that compare the digestibility of energy and nutrients in growing pigs fed the same diet from weaning to market. The objective of this experiment was, therefore, to determine if BW has an effect on the ATTD of energy and nutrients, and on the EPL and STTD of P in growing pigs between 10 and 140 kg.

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois.

Diets, Experimental Design, and Housing

Locally grown yellow dent corn from the 2009 crop year, soybean meal (**SBM**; Central Soya Company Inc., Gibson City, IL), and distillers dried grains with solubles (**DDGS**; One Earth Energy LLC., Gibson City, IL) were used in diet preparation (Table 4.1). Three semi-purified diets that contained SBM, DDGS, or corn as the sole source of P were formulated (Table 4.2). A P-free diet that was used to measure EPL was also formulated.

A total of 24 growing barrows that were the offspring of G-Performer boars mated to F-25 females (Genetiporc, Alexandria, MN) with an initial BW of 9.66 ± 0.67 kg were randomly

allotted to the 4 diets using a randomized complete block design with 6 replicate pigs per diet. Pigs were fed their assigned diets during five 12-d collection periods that started when they had an average initial BW of 9.66 ± 0.67, 22.29 ± 2.57, 53.77 ± 9.91, 92.73 ± 16.17, and 129.23 ± 18.55 kg. The P-free, SBM, and DDGS diets were prepared at the beginning of the experiment and stored at -20°C throughout the experiment. Due to storage limitations the corn diet was prepared in 2 batches; 1 batch was used for the initial 2 periods and the second batch was used for the remaining 3 periods. During collection periods, pigs were housed individually in metabolism cages that were equipped with expanded metal floors, a feeder, and a nipple drinker. A screen that allowed for total collection of feces was placed under each cage. Between collection periods, pigs were housed in grower pens with concrete slatted floors. A grower diet based on corn, SBM, and DDGS was provided on an ad libitum basis during these periods and water was available at all times.

Feeding and Sample Collection

During collection periods, pigs were fed 2.5 times their daily maintenance requirement for energy (i.e., 106 kcal ME/kg $BW^{0.75}$; NRC. 1998) in 2 equal meals and water was available at all times. The initial 5-d adaptation period was followed by a 5-d collection period. Chromic oxide was added to the morning meal on d 6 and ferric oxide was added to the morning meal on d 11 of each collection period. Fecal samples were collected from the appearance of chromic oxide to the appearance of ferric oxide according to the marker to marker approach (Adeola, 2001). Orts and refused feed were also collected from d 6 to 11. Fecal samples and feed refusals were stored at -20°C immediately after collection.

Data Recording and Chemical Analyses

The BW of each pig was recorded at the start and at the conclusion of each collection period. Fecal samples were dried in a forced air oven at 60°C prior to grinding. Samples were ground in a model 4, Thomas-Wiley laboratory mill with a 1mm screen (Thomas Scientific, Philadelphia, PA). Samples of corn, SBM, DDGS, diets, and feces were analyzed for DM (method 930.15; AOAC International, 2007) using an Isotemp 500 series gravity convection oven (Fisher Scientific, Pittsburg, PA) prior to ashing in an Isotemp muffle furnace (Fisher Scientific, Pittsburg, PA) for 6 h at 600°C (method 975.03; AOAC International, 2007). All samples were analyzed for P and Ca by inductively coupled plasma spectroscopy (method 985.01; AOAC International, 2007). Samples were also analyzed for ADF (method 973.18; AOAC International, 2007), NDF (Holst, 1973), CP (method 990.03, AOAC International, 2007), and for GE using bomb calorimetry (Model 6300, Parr Instruments, Moline, IL). Acid hydrolyzed ether extract (method 984.13, AOAC International, 2007) was also analyzed in all samples.

Calculations and Statistical Analyses

Values for ATTD, EPL, and STTD of P were calculated as described by Almeida and Stein (2010). The ATTD of GE, CP, ether extract, ash, ADF, NDF, and CA was calculated using the equation that was used to calculate the ATTD of P and values for DE were calculated by subtracting fecal energy from energy intake.

Homogeneity of variances among means were confirmed and outliers were identified using the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC) as values that had means that were more than 3 times the interquartile range (Devore and Peck, 1993). Data were analyzed using the MIXED procedure of SAS as a repeated measures analysis (Littell et al.,

1998) to determine effects of diet, BW, and the diet × BW interactions. The first-order autoregressive [AR(1)] covariance model was used for the analysis based on the Akaike Information Criterion. Orthogonal polynomial contrasts were used to determine linear and quadratic effects of BW on EPL and the digestibility of energy and nutrients. Coefficients for the contrast statements were derived using the interactive matrix language (IML) procedure of SAS. Significance and tendencies were set at $P \leq 0.05$ and $P \leq 0.10$, respectively. The pig was the experimental unit for all analyses.

RESULTS

One animal on the DDGS diet died during the first period and was not included in the data analysis. This animal was replaced by another pig of similar BW and genetic background for the remainder of the experiment. Proc UNIVARIATE identified 2 outliers. One pig on the SBM treatment was removed for the 10 kg-period and 1 pig on the DDGS treatment was identified as an outlier for the 130 kg-period and was removed before data were analyzed.

The main effect of diet was significant ($P < 0.01$) for all variables except Ca, and the main effect of BW was significant ($P < 0.01$) for all variables (Table 4.4). However, because of the significant diet × BW interactions ($P < 0.05$) for all variables except Ca and ether extract, main effects of diet and BW are not presented.

During the experiment, feed intake increased (linear, $P < 0.01$) for all diets as pig BW increased (Table 4.5). There was also an increase in the ATTD of P in SBM and corn as pig BW increased (linear, $P < 0.01$), but there was no effect of pig BW on the ATTD of P in DDGS. The EPL measured in mg/kg DMI was not influenced by pig BW, but if measured as mg/d, EPL increased (linear, $P < 0.01$) as BW increased, regardless of the diet being fed. The STTD of P in

SBM and corn increased (linear, $P < 0.01$) as pig BW increased, but for DDGS, no effect of pig BW on STTD of P was observed.

The ATTD of Ca in the DDGS diet decreased (linear, $P < 0.01$) and there was a tendency for a increase ($P = 0.06$) for the ATTD of Ca in the SBM diet as BW increased, but in the corn diet, no effect of BW on the ATTD of Ca was observed (Table 4.6). The digestibility of CP increased in SBM (linear and quadratic, $P < 0.05$), DDGS (linear and quadratic, $P < 0.01$), and in corn (linear, $P < 0.01$) as pig BW increased. The ATTD of ether extract in the SBM (quadratic, $P < 0.05$) and DDGS diets increased (linear, $P < 0.05$) as pig BW increased, but there was no effect of pig BW on the ATTD of ether extract in the corn diet. The digestibility of ash increased in the SBM and DDGS diets (quadratic, $P = 0.01$) and in the corn diet (linear and quadratic, $P < 0.01$) as pig BW increased.

There was no effect of pig BW on the ATTD of ADF in SBM, but the ATTD of ADF increased in DDGS and in corn (linear, $P < 0.05$) as pig BW increased. The ATTD of NDF in SBM tended to be greater for the 20, 50, and 90 kg pigs than for the 10 and 130 kg pigs (quadratic, $P = 0.07$), but in DDGS and in corn, the ATTD of NDF increased (linear and quadratic, $P < 0.05$ for DDGS; linear, $P < 0.01$ for corn) as pig BW increased. The ATTD of GE in the corn diets was not influenced by pig BW, but an increase in the ATTD of GE in the DDGS diet (linear and quadratic, $P < 0.01$) and in the SBM diet (quadratic, $P < 0.05$) was observed as BW increased. There was also an increase in DE of the SBM diet (quadratic, $P < 0.05$), in the DE of the DDGS diet (linear and quadratic, $P < 0.01$), and of corn (linear and quadratic, $P < 0.01$) as pig BW increased.

DISCUSSION

The values for ATTD of P in corn and SBM for the 10-kg pigs that were determined in this experiment are in agreement with values reported for 13-kg pigs and the ATTD values for P for the heavier weight groups are close to values for ATTD of P in corn and SBM with added microbial phytase (Almeida and Stein, 2010). The ATTD of P in DDGS was slightly greater than reported values between 50 and 68% (Pedersen et al., 2007a; Stein et al., 2009; Almeida and Stein, 2010), which indicates that a highly digestible source of DDGS was used in this experiment. Differences among sources of DDGS in P digestibility have been reported and may be a result of differences in the production of DDGS (Shurson et al., 2004; Pedersen et al., 2007a; Stein and Shurson, 2009).

There was no effect of BW on basal endogenous losses when they were calculated as mg/kg DMI. This is in agreement with observations for basal endogenous ileal losses of AA in pigs (Hess and Seve, 1999) and also in agreement with Petersen and Stein (2006) who reported that there is no difference in EPL for pigs from 27 to 78 kg. The linear increase in EPL as pig BW increased when calculated as mg/d is probably at least partially a result of increased feed intake as the pigs increased in BW. The average EPL (220 mg/kg DMI) that was determined in the current experiment is close to EPL values that have been reported for pigs between 10 and 30 kg (Stein et al., 2006; Widmer et al., 2007; Almeida and Stein, 2010). This observation confirms that EPL is not dependent on pig BW if measured as mg/kg DMI, which implies that EPL determined at a particular BW can be used for all weight groups of growing-finishing pigs. The fact that values that are relatively consistent are obtained among different experiments indicate that a constant value can be used to correct for basal endogenous losses of P. It may, therefore, not be necessary to measure EPL in all P digestibility experiments.

To our knowledge, the effect of BW on STTD of P has not previously been evaluated. However, Petersen and Stein (2006) determined the STTD of P in inorganic sources of P fed to pigs from 27 to 78 kg during 7 periods and they were not able to detect any effects of period on STTD, which indicates that within this weight range, STTD is not influenced by BW. The STTD of P in corn and SBM observed in this experiment for 10 kg pigs (26.4 and 47.6% respectively) are in agreement with data for 13 kg pigs (Almeida and Stein, 2010). The true total tract digestibility of P in SBM fed to 10-50 kg pigs has been reported between 41 and 59 % (Fan et al., 2001; Ajakaiye et al., 2003; Akinmusire and Adeola, 2009) and the present results also agree reasonably well with these data.

The linear increase in ATTD and STTD of P in corn and SBM was a surprising observation and to our knowledge, such an effect of BW has not been previously reported. It is well established that P is absorbed only in the small intestine of pigs (Bohlke et al., 2005; Dilger and Adeola, 2006). The present data, therefore, indicate that small intestinal digestion of P in corn and SBM increased as pig BW increased. The reasons for this increase were not elucidated in this experiment, but it is possible that as pigs become older, large bowel microbes enter the small intestine, because it has been shown that a proportion of dietary fiber is fermented in the small intestine (Urriola et al., 2010). The tendency for a quadratic increase in NDF digestibility of SBM and the linear increase in NDF digestibility in corn and DDGS indicates that fermentation increased as pig BW increased, and some of this fermentation may have taken place in the small intestine. Some of the microbes in the intestinal tract of pigs produce phytase (Lei et al., 1993a,b) and if these microbes enter the small intestine, it is possible that this can contribute to the increased digestibility of P as pig BW increased. The fact that there was no increase in the ATTD and STTD of P in DDGS supports this hypothesis because there is no effect of microbial

phytase on the ATTD and STTD of P in DDGS (Almedia and Stein, 2010). However, unlike DDGS, the majority of P in corn and SBM is bound in phytate, and phytase, therefore, increases the digestibility of P in corn and SBM.

The ATTD of Ca in the SBM diet concurs with recently reported values for a similar diet (Goebel and Stein, 2011). The increase in the ATTD of ash in the SBM and corn diets as pig BW increased is likely a result of the increase in the digestibility of P.

In this experiment, values for the ATTD of ether extract in DDGS concur with previous values (Stein et al., 2009). Because the corn and SBM diets contained both intact fat and added soybean oil, the ATTD for ether extract in these diets cannot be directly compared with previous values for the ATTD of ether extract in corn and soybean meal. The increase in ATTD of ether extract that was observed for the SBM diet and for DDGS was mainly a result of an increase from the first to the second period. This observation is in agreement with Cera et al. (1990) who reported that in weanling pigs, fat digestibility of soybean oil increased with BW.

An increase in CP digestibility in a mixed diet as pig BW increased was reported by Rodehutscord et al. (1999). In the present experiment, the ATTD of CP also increased with pig BW for all ingredients. The ATTD of CP in corn and DDGS concur with published values (Pedersen et al., 2007a,b; Stein et al., 2009).

Fiber is an anti-nutritional factor (NRC, 1998) and the high fiber content in DDGS may be one of the reasons for the low energy digestibility that has been reported (Stein and Shurson, 2009). Urriola and Stein (2010) reported that feeding a high fiber diet containing 30% DDGS for a prolonged time may result in an increase in the ATTD of ADF and NDF. In this experiment, both ADF and NDF digestibility increased linearly with BW for corn and DDGS.

The ATTD of ADF and NDF in DDGS and corn were also in agreement with previously reported values (Stein et al., 2009; Urriola et al., 2010). The fact that the ATTD of ADF and NDF did not increase with pig BW for SBM is likely a result of the presence of soluble dietary fiber in SBM, whereas corn and DDGS mainly contain insoluble fiber. Soluble fiber is easily fermented by pigs (Urriola et al., 2010), which is likely the reason for the relatively high ATTD of ADF and NDF in SBM.

The increased ATTD of GE in the DDGS diet as pig BW increased may be due to the increased fiber digestibility in DDGS. An increase in the ATTD of GE as pig BW increases is also in agreement with data from Noblet and van Milgen (2004). The ATTD of GE in corn is consistent with reported digestibility values ranging from 88.4 to 90% (Pedersen et al., 2007b; Goebel and Stein, 2011), but there was no effect of pig BW on the ATTD of GE in corn. This may be attributed to the lower fiber concentration in corn compared with DDGS and the ATTD of GE in the corn diet was relatively high in the smallest pigs used in this experiment, which left little room for improvement as pig BW increased.

The DE for corn that was measured in this experiment for the 3 lowest weight groups is within the range of previously reported values (NRC, 1998; Widmer et al., 2007; Baker and Stein, 2009). However the values for the 2 greatest weight groups are slightly greater than previous values. We are not aware of any other reports on the effect of BW on the DE of corn, but the increase in the DE of corn as pig BW increased that was observed in this experiment is in agreement with data reported for mixed diets (Noblet and van Milgen, 2004). The DE for the SBM and DDGS diets is the DE for the mixture of all the energy-containing ingredients in these diets, and these values can, therefore, not be directly compared with values for SBM and DDGS.

However, the increases in the DE that were observed for these diets as pig BW increased are in agreement with results for corn and other mixed diets (Noblet and van Milgen, 2004).

CONCLUSION

Results of the current experiment indicate that the digestibility of energy and some nutrients is affected by BW. There is a need for further research to elucidate the exact cause of this increase. It is possible that more than 1 digestibility value needs to be used for pigs in the weanling, growing, and finishing stages, but more research in this area is needed.

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Table 4.1. Analyzed composition of ingredients, as-fed basis

	Ingredient			
Item	SBM[1]	DDGS[1]	Corn A[2]	Corn B[2]
GE, cal/g	4,157	4,657	3,805	4,018
CP, %	47.12	26.57	7.58	7.43
DM, %	87.11	88.00	86.01	88.93
Ash, %	6.12	4.15	1.14	1.07
P, %	0.63	0.69	0.20	0.20
Ca, %	0.51	0.04	< 0.01	< 0.01
NDF, %	7.37	10.84	9.40	8.98
ADF, %	5.20	32.78	2.20	2.44
Ether extract, %[3]	1.69	10.57	2.61	3.61

[1]SBM = soybean meal; DDGS = distillers dried grains with solubles.

[2]Corn A was used for the diet fed to pigs during periods 1 and 2 and corn B was used for the diet fed to the pigs during periods 3, 4, and 5.

[3]Ether extract was analyzed as ether extract after acid hydrolysis (AOAC International, method 954.02).

Table 4.2. Ingredient composition of experimental diets (as-fed basis)

Ingredient	Diets			
	P-free	SBM[1]	DDGS[1]	Corn
Soybean meal	-	40.00	-	-
Distillers dried grains with solubles	-	-	40.00	-
Ground corn	-	-	-	97.10
Cornstarch	49.22	40.20	43.10	-
Sucrose	20.00	15.00	15.00	-
Soybean oil	4.00	3.00	-	1.0
Solka floc[2]	4.00	-	-	-
Ground limestone	0.80	1.10	1.20	1.20
Gelatin[3]	20.00	-	-	-
DL-Met	0.27	-	-	-
L-Thr	0.08	-	-	-
L-Trp	0.14	-	-	-
L-His	0.08	-	-	-
L-Ile	0.16	-	-	-
L-Val	0.05	-	-	-
Salt	0.40	0.40	0.40	0.40
Vitamin and mineral premix[4]	0.30	0.30	0.30	0.30
Potassium carbonate	0.40	0.40	-	-
Magnesium oxide	0.10	0.10	-	-

[1]SBM = soybean meal; DDGS = distillers dried grains with solubles.

Table 4.2 (cont.)

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

[3]Gelita Gelatine USA Inc., Sioux City, IA.

[4]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,128 IU; vitamin D3 as cholecalciferol, 2,204 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrocloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide and nicotinic acid, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Table 4.3. Analyzed composition of experimental diets (as-fed basis)

	Diet				
Item	P-free	SBM[1]	DDGS[1]	Corn A[2]	Corn B[2]
GE, cal/g	4,152	4,064	4,182	3,864	4,046
CP, %	23.39	18.87	10.86	7.43	7.60
DM, %	94.53	92.52	91.13	88.32	88.76
Ash, %	1.96	4.30	2.74	1.86	2.72
P, %	0.02	0.30	0.32	0.21	0.24
Ca, %	0.40	0.66	0.51	0.48	0.58
NDF, %	6.14	2.31	12.86	8.44	10.10
ADF, %	3.54	1.97	4.28	2.22	2.54
Ether extract, %[3]	2.47	3.11	4.45	3.85	4.09

[1]SBM = soybean meal; DDGS = distillers dried grains with solubles.

[2]Corn A was used for the diet fed to pigs during periods 1 and 2 and corn B was used for the diet fed to the pigs during periods 3, 4, and 5..

[3]Ether extract was analyzed as ether extract after acid hydrolysis (AOAC International, method 954.02).

Table 4.4. *P*-values for main effects and the interaction between diet and BW on energy and nutrient digestibility

	P-Value		
Item	Diet	BW	Diet × BW
ATTD of GE, %	< 0.01	< 0.01	< 0.01
DE, kcal/kg DM	< 0.01	<0.01	<0.01
ATTD of CP, %	< 0.01	< 0.01	< 0.01
ATTD of ether extract, %[1]	< 0.01	< 0.01	0.12
ATTD of ADF, %	< 0.01	< 0.01	0.02
ATTD of NDF,%	< 0.01	< 0.01	0.01
ATTD of P, %	< 0.01	< 0.01	< 0.01
STTD of P, %	< 0.01	<0.01	<0.01
ATTD of Ca, %	0.27	< 0.01	0.09
ATTD of ash, %	< 0.01	< 0.01	< 0.01

[1]Ether extract was analyzed as ether extract after acid hydrolysis (AOAC International, method 954.02).

Table 4.5. Effect of pig BW on feed intake and digestibility and basal endogenous losses of P in soybean meal (SBM), distillers dried grains with solubles (DDGS), and corn

	Initial BW, kg						*P*-value	
Item	9.6	22.3	53.8	92.7	129.2	SEM	Linear	Quadratic
SBM								
Feed intake, g DM/d	352	692	1,440	2,134	2,481	105	< 0.01	0.02
ATTD of P, %[1]	41.3	56.6	54.0	61.0	61.7	4.5	< 0.01	0.38
EPL, mg/d[2]	71.7	122.4	380.3	445.6	611.2	25.5	< 0.01	0.04
STTD of P, %[3]	47.6	61.9	62.2	67.5	69.3	4.5	< 0.01	0.27
DDGS								
Feed intake, g DM/d	293	615	988	1,497	2,368	105	< 0.01	0.08
ATTD of P, %	75.3	79.7	71.1	73.7	72.9	4.5	0.42	0.63
EPL, mg/d	60.3	108.7	261.1	312.6	574.5	25.5	< 0.01	0.06
STTD of P, %	81.2	84.6	78.6	79.7	79.9	4.5	0.57	0.70
Corn								
Feed intake, g DM/d	323	619	1,168	1,636	2,332	105	< 0.01	0.80
ATTD of P, %	17.7	33.3	43.6	55.1	61.2	4.5	< 0.01	0.11

Table 4.5 (cont.)

EPL, mg/d	66.6	109.4	308.7	341.5	553.0	25.5	0.01	0.10
STTD of P, %	26.4	40.6	50.5	60.5	67.6	4.5	< 0.01	0.18

[1]ATTD = apparent total tract digestibility.

[2]EPL = endogenous P loss. This value was calculated from pigs fed the P-free diet and was determined to be 206, 177, 264, 209, and 246 mg/kg DMI for pigs weighing 9.6, 22.3, 53.8, 92.7, and 129.3 kg, respectively. These values were not different ($P > 0.05$).

[3]STTD = standardized total tract digestibility. Values for the STTD of P were calculated by correcting ATTD values for basal endogenous losses of P.

Table 4.6. Effects of pig BW on apparent total tract digestibility (ATTD) of energy and nutrients in diets based on soybean meal (SBM), distillers dried grains with solubles (DDGS), or corn

	Initial BW, kg						*P*-value	
Nutrient	9.6	22.3	53.8	92.7	129.2	SEM	Linear	Quadratic
SBM								
ATTD of Ca, %	51.6	67.4	62.0	64.1	68.51	4.2	0.06	0.78
ATTD of CP, %	90.2	95.1	95.1	96.0	94.5	1.2	0.04	0.02
ATTD of ether extract, %[1]	76.1	83.4	83.2	85.6	81.0	2.4	0.25	0.04
ATTD of ash, %	65.0	78.1	74.7	77.9	70.5	2.9	0.58	0.02
ATTD of ADF, %	71.8	83.8	75.4	75.9	71.6	3.4	0.24	0.39
ATTD of NDF, %	72.3	81.4	77.0	77.2	69.8	2.9	0.15	0.07
ATTD of GE, %	94.0	96.4	96.0	96.4	95.1	0.6	0.56	0.02
DE, kcal/kg DM	4,130	4,234	4,216	4,236	4,177	26.8	0.57	0.02
DDGS								
ATTD of Ca, %	68.4	77.8	63.4	61.6	55.3	4.2	< 0.01	0.96
ATTD of CP, %	69.2	77.4	78.3	84.8	81.0	1.2	< 0.01	< 0.01
ATTD of ether extract, %	57.6	62.3	57.2	67.6	64.4	2.4	0.03	0.92

Table 4.6 (cont.)

ATTD of ash, %	48.4	66.0	60.6	65.4	58.4	2.9	0.23	0.01
ATTD of ADF, %	56.1	63.3	64.1	70.6	66.4	3.4	0.03	0.16
ATTD of NDF, %	50.9	56.9	60.6	67.7	62.3	2.9	< 0.01	0.03
ATTD of GE, %	83.5	86.1	85.8	89.5	86.9	0.6	< 0.01	< 0.01
DE, kcal/kg DM	3,830	3,950	3,928	4,106	3,985	26.8	< 0.01	< 0.01
Corn								
ATTD of Ca, %	62.5	72.7	64.4	71.0	71.4	4.2	0.33	0.87
ATTD of CP, %	74.9	80.3	76.8	84.2	82.1	1.2	< 0.01	0.41
ATTD of ether extract, %	60.9	66.0	56.1	69.5	65.5	2.4	0.14	0.45
ATTD of ash, %	22.5	48.5	56.8	67.5	62.3	2.9	< 0.01	< 0.01
ATTD of ADF, %	47.1	56.9	39.1	61.1	58.5	3.4	0.03	0.13
ATTD of NDF, %	52.4	60.6	52.0	66.0	63.3	2.9	< 0.01	0.84
ATTD of GE, %	88.4	89.9	86.9	91.2	89.4	0.6	0.13	0.68
DE, kcal/kg DM	3,838	3,904	3,960	4,157	4,074	26.8	< 0.01	< 0.01

[1]Ether extract was analyzed as ether extract after acid hydrolysis (AOAC International, method 954.02).

CHAPTER 5

CONCLUSION

The combination of the 3 experiments resulted in new information that will need to be applied to the swine industry. In the first 2 experiments, the not a correlation between the relative bioavailability method using a slope ratio technique and the standardized total tract digestibility (**STTD**) values is further proof that the STTD method is a valid methodology in conduction of digestibility studies. This will allow the industry as a whole to move away from the bioavailability numbers currently published and toward a digestibility system. This change will allow for a more accurate calculation of the P concentration in diets fed to pigs by allowing researchers to formulate diets using values for the individual feed ingredients. This will in turn allow for more accurate calculation of diets if there are numerous ingredients in the ration.

Experiment 3 further evaluated the STTD and ATTD of various nutrients in common feed ingredients through the life of the pig. Even though the findings were in direct contradiction with the limited previous research there is a scientific explanation as why the digestibility changed with increasing BW for soybean meal and corn, but not for distillers dried grains with solubles. In the future, there is a need for further research in the area to determine why the digestibility changed.

Overall, the 3 experiments add to the already expansive knowledge of the apparent total tract digestibility of nutrients and further confirm the accuracy of the STTD method. Moreover, the findings lead to the conclusion that values for STTD of P is a better parameter for evaluating

individual ingredients for nutrients both because of its accuracy and cost. The values for the STTD of P in ingredients will be the future of swine diet formulation.