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STANDARDIZED TOTAL TRACT DIGESTIBILITY OF PHOSPHORUS IN RICE BRAN WITH AND WITHOUT PHYTASE SUPPLEMENTATION IN SWINE DIETS

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JERUBELLA JERUSALEM ABELILLA

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The thesis attached hereto, entitled "**STANDARDIZED TOTAL TRACT DIGESTIBILITY OF PHOSPHORUS IN RICE BRAN WITH AND WITHOUT PHYTASE SUPPLEMENTATION IN SWINE DIETS**" prepared and submitted by **JERUBELLA JERUSALEM ABELILLA** in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (ANIMAL SCIENCE)** is hereby accepted.

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BIOGRAPHICAL SKETCH

The author is the eldest among four children born to Mr. Diomedes E. Abelilla and Mrs. Betty J. Abelilla on December 22, 1987 at Calantipayan, Lopez, Quezon.

She finished her elementary education at New Jerusalem School in 2000 and secondary education at Lopez National Comprehensive High School in 2004. In 2008, she obtained a Bachelor of Science in Agriculture, major in Animal Science, with specialization in Animal Nutrition from the College of Agriculture, University of the Philippines, Los Baños. In same year, she took and passed the Licensure Exam for Agriculturist.

Because of her passion for research particularly in animal nutrition, she joined a feed milling company as a research assistant in November 2008. After almost two years, she decided to pursue an MS degree major in Animal Science with specialization in Animal Nutrition, minor in Biochemistry.

In June 2010, she was awarded a DOST-SEI graduate scholarship under the Accelerated Science and Technology Human Resource Development Program being implemented by Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development.

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ABSTRACT

ABELILLA, JERUBELLA JERUSALEM, University of the Philippines Los Baños. April 2014. **Standardized Total Tract Digestibility of Phosphorus in Rice Bran With and Without Phytase Supplementation in Swine Diets.**

Major Professor: **Dr. SONIA P. ACDA**

Two studies were conducted to determine the standardized total tract digestible (STTD) phosphorus (P) in rice bran with corn and soybean meal (SBM) as reference ingredients. The objectives of the first study were to determine the STTD P values in the ingredients with and without phytase when fed to growing pigs and estimate P release using *in vitro* procedure. The second study aimed to determine the effects of diets formulated with the same level of STTD P on the growth performance of growing pigs regardless of total and available P level. In study 1, 36 barrows (PIC L337 × C24, initial BW = 22.3 ± 1.4kg) were randomly allotted to six semi-purified diets with each ingredient as the sole source of P with and without phytase supplementation. Each pig was housed in metabolism cages that allowed total collection of feces. In study 2, the same set of animals (initial BW = 33.0 ± 2.7 kg) were randomly allotted to four corn-SBM based diets following a 2 x 2 factorial in a randomized complete block design. Factors were phytase (0 and 500FTU/kg) and rice bran (0 and 10%), with initial body weight as a blocking factor. All diets were formulated to contain 0.31% STTD P and fed to growing pigs for 28 d. Results in study 1 showed that addition of phytase increased the

STTD of P in corn from 53.38 to 73.35% (P<0.005), in SBM from 46.12 to 73.05% (P<0.05), and in rice bran from 49.76 to 64.43% (P<0.05). For the *in vitro* release of P experiment, the in vitro release of P was highly correlated (r = 0.94) with the in vivo P digestibility. Linear regression equation showed that for every additional 1% in *in vitro* P release, the *in vivo* STTD of P is expected to increase by an average of 0.7869%. In study 2, there was no (P > 0.05) phytase × rice bran interaction in all the growth parameters measured. There was also no (P > 0.10) difference in ADG, ADFI, G:F and final BW between pigs diets with and without phytase and diets with 0 or 10% rice bran. In conclusion, addition of phytase improved the STTD of P in rice bran. The regression equation obtained in this study could be used to estimate the *in vivo* STTD of P. The growing pig diets can be formulated based on STTD of P. Supplementation of inorganic P can be reduced or eliminated without reducing animal performance when using phytase, rice bran or combination of phytase and rice bran in the diet.

INTRODUCTION

After energy and amino acids, phosphorus is the next most expensive nutrient to provide in swine diets (NRC, 1998). This can be attributed to poor availability of P in plant sources due to lack of phytase enzyme in monogastric animals. To alleviate this condition, animal nutritionists resort to supplementation of inorganic source of phosphorus, which results in increased feed cost and potential source of environmental pollutants. Thus, a lot of effort had been made to utilize phosphorus sources efficiently. This includes the use of endogenous phytase, an enzyme that catalyzes the release of phosphorus bound to phytic acid in different plant sources, and the continuous research on more accurate evaluation system of phosphorus bioavailability in various ingredient sources.

The system currently used in evaluating phosphorus in feed ingredients is the relative bioavailability (RBV, %) or available phosphorus (NRC, 1998). However, aside from inconsistent results of assay and influence of the assay criteria used (Fan *et al*., 2001). RBV values are not additive in mixed diets. This means the sum of RBV phosphorus values from two or more P sources in the diet does not equate to the actual available phosphorus of the whole diet. This could lead to either under- or overestimation of the phosphorus in the raw materials resulting in poor animal performance or environmental problems, respectively.

The evaluation system used in Europe is the digestible phosphorus. This directly measures the absorbed and excreted portion of phosphorus in raw materials by pigs. Unabsorbed P can be measured either by collecting digesta from the ileum (apparent ileal digestibility, AID) using cannulation technique or by evaluating P excreted in the feces of animal (apparent total tract digestibility, ATTD). Several researches showed that there was no significant difference in the digestibility values of P when using either AID or ATTD (Bohlke *et al*., 2005; McGinnis *et al*., 2007), thus the latter is a more practical and an easier technique to use. Apparent total tract digestibility (ATTD) is a system that accounts for the P excreted in the feces of animal given a specific ingredient. However, ATTD does not account for the basal endogenous losses of P (EPL), thus underestimating the digestibility of P. The basal endogenous losses are produced in response to the dry matter intake but independent of the material being fed (Stein, 2011). The digestibility values of phosphorus in this system are not additive when formulating diets.

Standardized total tract digestibility (STTD) of phosphorus is a more accurate system of evaluating phosphorus digestibility as it accounts for the basal EPL and is more additive in mixed diet compared to ATTD of phosphorus (Almeida and Stein, 2010). Hence, US NRC recommends the use of STTD of phosphorus instead of available phosphorus (Sulabo, 2011) when formulating diets.

Another approach in minimizing feed cost is the use of alternative ingredients that are readily available and at relatively cheaper cost. The use of RBD_1 in swine diets is very common in the Philippines and other Asian countries. Aside from good energy and

amino acid source, RBD_1 contains a significantly higher amount of P compared to corn and SBM. However, to our knowledge, there are no actual values for the STTD of P in RBD_1.

Objectives of the Study

The general objective of the study was to determine the STTD of phosphorus in rice bran with and without phytase in pigs. More specifically, this study aimed:

Study 1

1. to determine and compare the ATTD and STTD of phosphorus in rice bran, soybean meal (SBM) and corn by growing pigs;
2. to determine the effect of phytase supplementation on the ATTD and STTD of phosphorus in rice bran, SBM and corn by growing pigs;
3. to determine phosphorus release from rice bran, SBM and corn with or without phytase using *in vitro* procedure; and
4. to evaluate whether the *in vitro* procedure can be used to estimate *in vivo* phosphorus release in rice bran, soybean meal and corn with or without phytase.

Study 2

1. To validate the effect of diets formulated based on STTD of phosphorus on the growth performance of growing pigs; and
2. to evaluate feed cost efficiency of diets with rice bran and with phytase supplementation.

Time and Place of the Study

The animal experiment was conducted at the University Animal Farm from September 18 to December 7, 2012 at the Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines Los Baños, College, Laguna.

REVIEW OF LITERATURE

Phosphorus

Phosphorus occurs in the forms, namely, hydroxyapatite in calcified tissues; phospholipids, which are major components of most biological membranes; and nucleotides and nucleic acids (Cashman and Flynn, 1999, as cited by Sulabo, 2003).

Functions. Phosphorus is an important mineral in the body as it is involved in different processes such as component of the bone, energy storage and transfer, component of nucleic acids and prevents the leakage of biochemicals from the cell (Brody, 1999). About 85% of the body's phosphate occurs in bones, with 14% in soft tissues and about 1% in the extracellular fluids. Deficiency in phosphate causes decrease in bone mineralization (Guyton and Hall, 2006). Phosphorus is also needed in energy storage and transfer. Adenosine triphosphate (ATP) is produced from the combustion of carbohydrates (glycolysis and citric acid cycle), fatty acids (beta-oxidation), and proteins (requires hydrolysis into amino acid, degradation into citric acid intermediate, and finally into acetyl coenzyme A and carbon dioxide. It also provides energy to different activities in the body such as synthesis of most important cellular components (peptide linkage, cholesterol, hormones, among others), muscle contraction, active transport across membranes, glandular secretion, and nerve conduction (Guyton and Hall, 2006). According to Brody (1999), 25% of the ATP synthesized per day is used by the sodium pump (Na, K-ATPase).

Phosphorus is a component of nucleic acids. The phosphate group links two adjacent nucleosides in the deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Phosphate group has the ability to bind two organic molecules through its hydroxyl groups, and still remain negatively charged (Westheimer, 1987). This negativity of phosphate is important as it stabilizes the polymer by protecting it from attack by hydroxide ions that would lead to hydrolytic cleavage (Brody, 1999; Berg *et al*., 2007). Nucleic acids need to be stable to prevent degradation of the sequence information they contain that would be passed from one generation to another (Berg *et al*., 2007).

Phosphate groups also prevent phosphorylated compounds, such as nucleotides, intermediates of glycolysis, and vitamin B_6 from leaking out of the cell. The phosphate group makes the compound more hydrophilic, thus preventing them from passing through the lipophilic part of the membrane (Brody, 1999).

In cells and tissues, phosphate and bicarbonate buffer systems maintain intracellular and extracellular fluids at their optimum (physiological) pH, which is usually close to pH 7. Enzymes generally work optimally at this pH (Nelson and Cox, 2004). Phosphate is the main anion of intracellular fluid. It is capable of combining reversibly with many coenzyme systems and with multiple other compounds needed for different metabolic processes (Guyton and Hall, 2006).

Phosphate Turnover. Phosphate is absorbed throughout the small intestine. Phosphate absorption in the duodenum requires sodium-dependent transport mechanism, which is enhanced by calcitriol (1, 25 dihydroxycholecalciferol); whereas it is passively

absorbed in the jejunum and ileum, which is dependent on the concentration of phosphate in the lumen and independent of other nutrients and energy-using processes. Approximately 200 mg of phosphorus is excreted per day in fluids of the gastrointestinal tract, wherein two-thirds is reabsorbed by the gut (Brody, 1999). Inorganic phosphate is absorbed faster, but tends to be excreted in the urine rather than to be used by the tissues (Schuette and Linkswiler, 1982).

The rate of phosphorus renal excretion depends on the plasma concentration of phosphorus. When phosphorus concentration in the plasma is below the critical level of 1mmol/L, all of the phosphorus in the renal tubules is reabsorbed. The extracellular fluid concentration of phosphate is controlled by the kidneys by altering phosphate excretion according to the plasma phosphate concentration and the rate of phosphate filtration (Guyton and Hall, 2006). Another factor that increases phosphate excretion is the release of parathormone (PTH). PTH is secreted when there is low amount of calcium in the plasma to maintain calcium homeostasis, which in effect increases renal excretion of phosphate to enhance the ionization of plasma calcium by lowering the $[Ca^{2+}] \times [PO_4^{-3}]$ solubility product (Hadley, 2000). When PTH is released, reabsorption of renal phosphate is inhibited, resulting to hypophosphatemia, which in turn stimulates the release of calcium from the bone or prevents calcium phosphate precipitation in tissue (Despopoulos and Silbernagl, 2003).

Phytate

Phosphorus from plant sources has generally low availability since majority of phosphorus is bound to phytic acid (salt form, phytate). Phytate (myo-inositol hexaphophate) is composed of an inositol ring with six phosphate groups, as shown in Figure 1 (Woyengo *et al*., 2008). Phytate is the primary storage form of phosphorus in plants but cannot be utilized by monogastric animals since they lack the enzyme phytase needed for its degradation. Due to poor utilization of phosphorus in plant sources, nutritionists resort to supplementation of inorganic phosphate to satisfy the requirement of animal, which sometimes is not economical given its high price; and environmentally hazardous as it leads to excess supplementation of phosphorus. When excess phosphorus is excreted and reaches the bodies of water, it leads to eutrophication, a phenomenon exhibited by excessive plant growth due to excess phosphorus and nitrogen in the water (Gross and Boyd, 1998), thus depleting oxygen and causing morbidity and mortality of the aquatic organisms.

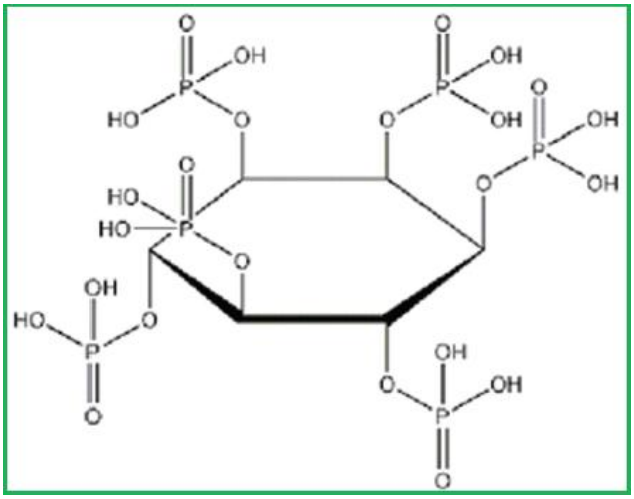

Figure 1. Structural formula of phytate molecule.

Aside from decreasing phosphorus bioavailability, phytic acid can also form insoluble complex with almost all multivalent cations, such as calcium, zinc, iron and copper, which are insoluble above pH 6 to 7 (Cheryan and Rackis, 1980); and with amino acids and starch, making them resistant to digestion, thus decreasing the bioavailability of the said nutrients. Hence, phytase was developed to address problems associated with phytate.

Phytase

One of the significant milestones in animal nutrition is the development and acceptance of phytase to reduce phosphorus excretion (Cromwell, 2009). Among the advantage of the inclusion of this enzyme in the diet are improvement in phosphorus digestibility and reduction in fecal phosphorus (Jongbloed *et al*., 1992 and Cromwell *et al*., 1993, cited by Cromwell, 2009), improved performance of animals, reduced formula

cost and decreased phosphorus discharge. As a result, the use of inorganic phosphates in the diet may be reduced or completely removed.

Phytase (myo-inositol-1,2,3,4,5,6-hexakisphosphate phosphohydrolase) is a kind of phosphatase that hydrolyzes phosphoester bond and removes phosphate groups in phytate molecule one by one producing the end products inositol and inorganic phosphate. Dephosphorylation of the phytate molecule occurs at different reaction sites (3-phytase or 6-phytase).

Phytases can be derived from animals, plants, or microbial sources (Pallauf and Rimbach, 1997). However, animals produce only small and inadequate amounts of the phytase (Patience and DeRouchey, 2004). Also, plant ingredients produce low concentration of phytase. Microbial phytase have been in practical use for over a decade. According to Almeida (2010), microbial phytases are categorized according to the site of hydrolysis of phosphate group in the inositol ring of phytic acid, namely 3-phytases, 6-phytases and 5-phytases. For example, 3-phytases (*Aspergillus niger*) initially hydrolyze phosphate group at the third carbon, while 6-phytases such as *Escherichia coli* initiate hydrolysis of phosphate at sixth carbon, and 5-phytases (*Pisium sativum*) initiate dephosphorylation at the fifth carbon of inositol (Rao *et al*., 2009, cited by Almeida, 2010). After releasing the first phosphate group, the five remaining phosphate groups can be sequentially released from phytic acid by phytase.

Numerous studies have been conducted proving the efficacy of phytase in the availability of phosphorus from plant sources. Efficacy of supplementation is dependent

on the microbial source, form of the enzyme, temperature and pH optima of the enzyme, diet mineral concentration (Ca, Fe, Mg, Cu, and Zn), ingredients used in the diet, diet manufacturing methodology, form of the diet, location of addition of phytase, type and level of vitamin D metabolites, disease status of the animal and other factors (Ravindran *et al*., 1995).

Each phytase has different recommendations regarding digestibility improvements for phosphorus, calcium, amino acids, and energy. One possible explanation for the different responses in amino acid digestibility may lie in the different effects of these phytase sources on endogenous amino acid flows. Also, phytases differ in biochemical and biophysical properties such as the optimum pH and the ability to resist hydrolysis within the digestive tract.

The differences in the biochemical and biophysical properties of phytase and the pH of the gut from which the phytate complex was liberated may lead to different levels of nutrients being released in response to different phytases. Reviewed literatures indicate that 500FTU/kg of enhanced *E.coli* phytase can release 1.3kg/t phosphorus while the equivalent values for the standard *E.coli*, *Aspergillus* and *Peniophora* phytases are 1.2, 1.0 and 0.67 kg/t respectively.

A lot of research have been conducted demonstrating improvement on the ATTD and STTD of P of corn-SBM based diets (Geraets *et al*., 2005), SBM (Fan *et al*., 2001; Goebel and Stein, 2011; Rojas and Stein, 2012), corn (Almeida, 2010), and field peas (Stein, 2006).; but showed no improvement on STTD of P when phytase was added on

distillers dried grains with soluble (DDGS) (Almeida and Stein, 2010). To our knowledge, no actual evaluations on the ATTD and STTD of P in rice bran are available yet.

Rice Bran

Rice bran is an alternative feed ingredient that appears to be one of the best sources of energy as it is cheaper and abundantly available in Asian countries (Soren *et al*., 2003). On the average, RBD_1 contains 12.14% crude protein, 13.79% crude fat, 5.27% crude fiber, 3000 kcal (ME for swine), 0.15% calcium and 1.48% total phosphorus (PHILSAN, 2010). According to INRA-AFZ (2004), RBD_1 contains 1.61% total phosphorus, wherein 85% is bound in the form of phytate.

The nutrient profile of RBD_1 makes it one of the most valuable alternative ingredients for both swine and poultry diets in rice producing countries. It can be fed to almost every stage of swine up to a certain level except in booster pigs. Younger piglets have underdeveloped gastrointestinal tract thus requires more digestible feed ingredients. For nursery pigs, 10% of rice bran can be included in the diet to improve feed palatability and to decrease the incidence of diarrhea caused by factors other than bacteria (Attamangkune, 2007).

For growing-finishing rations, Calvert *et al*. (1985) observed a decrease in feed consumption when 40% rice bran was included in corn-SBM diet, and thus recommended up to 25% inclusion of rice bran. The same level was recommended by Attamangkune

(2007), wherein RBD_1 can be included up to 25% in finishing diets without affecting animal performance but should not be more than 20% of the ration to avoid soft pork. Due to high level of linoleic acid, RBD_1 is prone to rancidity. Rancid RBD_1 must not be included in finishing diets as it has negative effect on growth performance and pork quality of finishing pigs (Chae and Lee, 2002).

For breeders, Attamangkune (2007) recommends at least 10% of rice bran to be included in gestating and lactating diets to improve palatability and laxative effects of feeds. Soren *et al*. (2003) concluded that rice bran can be included up to 41% in breeder (gilts) diets replacing 50% of maize and wheat bran.

Because of the above mentioned problems associated with the use of RBD_1, PHILSAN (2010) recommends maximum inclusion rate of 7% in pre-starter diets, 10% in starter diets, 20% in grower diets, and 30% in finisher, breeder and lactating diets.

Evaluation of Phosphorus Bioavailability

Relative bioavailability (RBV) or Slope-ratio assay

Phosphorus and calcium requirement of animals were established using the bone ash and bone breaking strength as response criteria being very sensitive to calcium and P adequacy (Cromwell, 2005). Cromwell (1979) of the University of Kentucky developed the slope-ratio assay in determining the relative bioavailability of phosphorus in feedstuffs that served as the basis for available P**.** This is a combined estimation of

digestive and post-absorptive utilization of P at the tissue level. This method gives relative biological values for a particular ingredient on a conventional scale. Monosodium phosphate (relative value = 100) is commonly used as reference source, while some studies used monocalcium phosphate (NRC, 1998). However, P digestibility of monosodium phosphate is about 85 to 90% (INRA-AFZ, 2004), thus bioavailability estimates of feed ingredients must be adjusted with the reference standard. Response criteria commonly used were blood and bone parameters, wherein the former is less sensitive to the amount of absorbed P, while the latter is more sensitive to increasing P availability (Sulabo, 2011). Also, NRC (1998) did not distinguish the reference used in determining bioavailability of P. There were studies that estimated bioavailability values using monocalcium phosphate, while some used monosodium phosphate, which had different bioavailability values of 92 and 83%, respectively. Hence, among the disadvantages of this evaluation system includes inconsistent assay results and variable assay criteria used (Fan *et al*., 2001); RBV values are not additive in mixed diets; and relatively expensive procedure of biological assay (Sulabo, 2011). Hence, the new NRC (2012) for swine changed from available phosphorus to digestible phosphorus. Table 1 shows the comparison of available and digestible phosphorus of different ingredients. Fish meal, dried whey, DCP and MSP had relatively lower digestible phosphorus values than available phosphorus values, which means the amount of phosphorus for these ingredients is overestimated when formulating based on the available phosphorus values which could result to poor performance of animals. On the other hand, corn, SBM and

meat and bone meal, had higher digestible P than available P, which means bioavailable P for these ingredients were underestimated, leading to excess supplementation of P in formulated diets. This could lead to more expensive rations and higher risk for environmental pollution.

Table 1. Availability and digestibility of P in different ingredients (Sulabo, 2011).

INGREDIENT	TOTAL P,%	RBV OF P, %	% AVAIL P	ATTD OF P, %	% DIG P
Corn	0.30	14	0.04	20	0.06
Wheat	0.35	49	0.17	47	0.17
Soybean meal	0.65	31	0.20	40	0.26
Meat and bone meal	6.00	67	4.02	80	4.80
Fish meal	2.50	94	2.35	86	2.00
Dried whey	0.80	97	0.78	82	0.66
Dicalcium phosphate	18.00	107	18.00	67	12.00
Monosodium phosphate	22.00	100	22.00	85	18.50

Apparent total tract digestibility (ATTD)

McGinnis (2007) conducted experiment on swine and determined the duodenal, ileal and total tract digestion of P in corn-SBM diet containing 0, 500, and 1000 units of phytase. Results showed lower values of digestible P from the duodenum than those obtained from ileal and total tract, and there was no significant difference between the values obtained from ileal and total tract digestibility. This confirmed the results obtained

by Bohlke *et al.* (2005) that showed no significant difference in apparent ileal digestibility (AID) vs ATTD of phosphorus values obtained from low-phytate corn, normal corn and SBM. The same result was observed by Geraets *et al*. (2005) wherein he observed that the apparent duodenal digestibility (ADD) of P was lower ($P<0.05$) than AID and ATTD of P in corn-SBM diets with and without phytase, but AID and ATTD of P values were not different in both diets. This means that P is only absorbed in the SI and none in the large intestine.

However, several studies showed that increasing P level in the diet also increases the ATTD of P values per ingredient. This was observed by Fan *et al*. (2001) wherein increasing SBM inclusion level also increases ATTD of P values. Another study was ATTD does not account for the endogenous losses of phosphorus excreted in the feces together with the undigested phosphorus from the diet. Thus, phosphorus digestibility is underestimated which may consequently result to excess phosphorus in the diet. Fan *et al*. (2001) observed that increasing SBM inclusion level also increases ATTD values.

Determination of endogenous losses. Total nutrient in the fecal output is divided into two components. The first component includes the undigested nutrients from the feed. These are the nutrients in the raw materials that remained undigested due to several reasons. The second component is composed of the endogenous nutrients secreted in response to feed intake and part of other metabolic processes inside the body of animals. For the total endogenous P losses (EPL), it is further divided into two components: the basal EPL and the specific EPL. Basal EPL are produced in response to dry matter intake,

while specific EPL may be induced by dietary factors such as high concentrations of fiber or anti-nutritional factors (Petersen and Stein, 2006) thus may vary among different ingredients. Total EPL can be measured using regression analysis or using ingredients with radioactive labeled P. This value can be used to correct ATTD P to calculate true total tract digestible (TTTD) P. However, it is difficult to measure the total EPL accurately and consistently using these procedures, as observed in wide range of values from 8 to 670 mg/kg dry matter intake (Akinmusire and Adeola, 2009; and Shen *et al*., 2002, as cited by Stein, 2011).

On the other hand, basal EPL can be measured by providing P-free diet (Petersen and Stein, 2006). Several studies have been conducted to determine basal EPL and reported values ranging from 139 to 219 mg/kg DMI (NRC, 2012). Stein (2011) concluded that there is no need to determine the basal EPL in every experiment since previous studies showed small variability among values and only ATTD of P is necessary to be determined. ATTD can be corrected using the average value of basal EPL which is 200 mg per kilogram dry matter intake to calculate STTD (Stein, 2011).

Almeida and Stein (2010) demonstrated that STTD of P is additive in mixed diets and concluded that diets can be formulated based on STTD of P without affecting growth performance.

Standardized total tract digestibility (STTD)

STTD accounts for the endogenous phosphorus losses (EPL) and is independent on the amount of phosphorus included in the diet, thus additive in mixed diet (Stein, 2011)**.** ATTD of P depends on the inclusion rate of P in the diet (Fan *et al*., 2001).

According to Stein (2011), proportion of endogenous losses of P in the total P output is inversely proportional to the inclusion rate of P in the diet. When P inclusion in the diet is low, the proportion of endogenous losses of P is greater in the total P output. Thus, ATTD requires to be corrected for endogenous losses.

In Vitro Procedure to Determine Phosphorus Release from the Diet

In vitro procedure to estimate enzymatic phosphorus release in corn-SBM swine diet was developed by Liu *et al* (1997) as shown in Figure 2. The procedure basically simulates the environment in the stomach and small intestine of swine, wherein microbial dephosphorylation and mineral absorption occur.

For stomach simulation, a pH value of 2.5 was chosen because it is within gastric pH range of growing pigs (Moore and Tyler, 1955a, b; Lawrence, 1972; Argenzio and Southworth, 1975; cited by Liu *et al*., 1997) and is one of the two optimum pH for microbial phytase activity (Simons *et al*., 1990).

For the dialysis medium, a pH of 6.0 was chosen because it is the estimated pH level of digesta in the small intestine (Moore and Tyler, 1955a, b; Hartman *et al*., 1961; Argenzio and Southworth, 1975; cited by Liu *et al*., 1997).

An in vitro procedure for determining dephosphorylation from the diet is considered valid if it has a high recovery rate of added inorganic phosphorus, wherein Liu *et al*. (1997) study averaged 98%. The said procedure was also able to predict P release from corn-SBM diets, predicts effectiveness of microbial phytase on dephosphorylation of phytate in corn-SBM diets, and is sensitive to dietary calcium level. Also, this procedure significantly correlated with the growth performance ($r > 0.998$) and P digestibility ($r = 0.999$) of growing pigs given diets with 0.32% total phosphorus as supplemented with microbial phytase at 0, 250, or 500 PU/kg of diet (Liu *et al*., 1997).

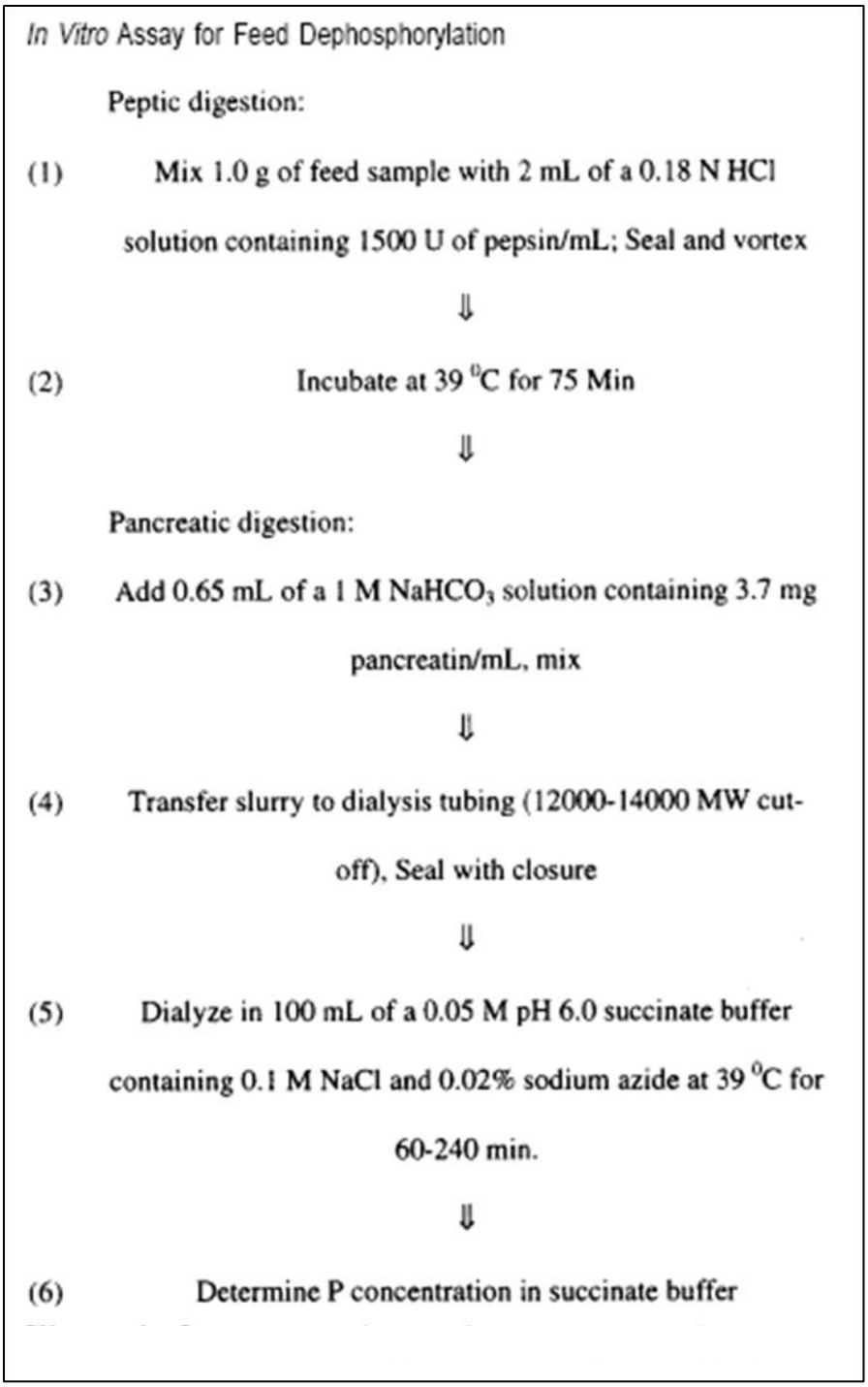

Figure 2. In vitro procedure in determining P release (Liu *et al*., 1997).

MATERIALS AND METHODS

Study 1. Effect of Phytase on the STTD of Phosphorus in Rice Bran

Experiment 1. In vivo procedure to determine the effect of phytase on the STTD of P in corn, SBM and RBD_1.

Experimental Design

A total of 36 barrows (PIC L337 x C24, initial BW = 22.3 ± 1.4kg) were randomly allotted to corn, SBM and RBD_1 semi-purified diets with and without phytase in a randomized complete block design, with body weight as the blocking factor. Six individually penned pigs were assigned to each of the six formulated diets.

Experimental Diets

Corn and SBM served as reference ingredients for the validation of results with RBD_1 since the STTD P of the said ingredients were already established. The diets were formulated by mixing cornstarch and brown sugar with each of corn, SBM and RBD_1. Three additional diets identical to the initial diets were also formulated with the exception that 500 units of phytase were added. The test ingredients (corn, SBM and RBD_1) were the sole source of phosphorus in 6 diets. Vitamins and all minerals except phosphorus were included in the diets according to current requirements (NRC, 1998). Purified diets were formulated using cornstarch, brown sugar and oil as source of energy. Ingredient

composition and calculated nutrient content of semi-purified diets (as-fed basis) are shown in Table 2.

Management of Experimental Animals

Pigs were fed their experimental diets for 10 days. The first 5 days was considered an adaptation period to the diet. Pigs were fed twice daily. The amount of feed per day given to the animals was 3 times the maintenance energy requirement of the animal (106kcal ME/$kg^{0.75}$; NRC, 1998). The amount of feed offered and refusals were recorded to determine the actual feed intake of individual animal. Water was made available at all times.

Digestion Trial

Feces were collected from day 6 to 10 according to marker to marker approach (Adeola, 2001), wherein marker was used to determine the beginning and the conclusion of fecal collections. In this experiment, chromic oxide was used as a marker. On the first (morning) meal of the 6th day of experiment, 1g of chromic oxide was added into 100 g of the diet. After the feed with the marker was consumed, the remaining feed allotment without the marker for that day was given to the animals. Collection of feces started when fecal matter appeared green. On day 11, 2g of chromic oxide was again fed, as was done on day 6. The inclusion of chromic oxide was increased from 1g to 2g to better distinguish the feces to be collected on the said experiment. Fecal collection ended upon the first appearance of the marker. Fecal matters were weighed daily, pooled and samples

Table 2. Ingredient composition and nutrient content of semi-purified corn/SBM/ RBD_1-based diets.

INGREDIENT, %	CORN		SBM[1]		RBD_1[2]	
Phytase, FTU/kg[3]:	0	500	0	500	0	500
Ingredients						
Corn yellow (local)	96.77	96.77				
Soybean meal, US high			40.00	40.00		
Rice bran D_1					50.00	50.00
Corn starch	0.21	0.19	47.28	47.26	26.98	26.96
Brown Sugar			10.00	10.00	20.00	20.00
Vitamin Concentrate[4]	0.02	0.02	0.02	0.02	0.02	0.02
Mineral Premix[5]	0.10	0.10	0.10	0.10	0.10	0.10
Coconut oil	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	1.50	1.50	1.20	1.20	1.50	1.50
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Phytase[6]		0.025		0.025		0.025
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Analysis						
Crude Protein (Nx6.25), %	7.79	7.79	18.80	18.80	6.07	6.07
Standardized Ileal Digestible Lys: ME, g/Mcal	0.61	0.61	3.07	3.07	0.60	0.60
Metabolizable Energy, kcal/kg	3293	3292	3393	3392	3335	3334
Standardized Ileal Digestible Amino Acid[6], %						
Lysine	0.20	0.20	1.04	1.04	0.20	0.20
Methionine	0.15	0.15	0.25	0.25	0.10	0.10
Methionie+Cystine	0.32	0.32	0.50	0.50	0.19	0.19
Threonine	0.23	0.23	0.63	0.63	0.16	0.16
Tryptophan	0.06	0.06	0.23	0.23	0.05	0.05
Calcium, %	0.70	0.70	0.62	0.62	0.62	0.62
Total Phosphorus	0.25	0.25	0.27	0.27	0.74	0.74

[1]SBM = soybean meal
[2] RBD1 = rice bran D1
[3]FTU = phytase units.
[4]The vitamin premix provided the following quantities of vitamins per kilogram of complete diet: Vitamin A, 10, 000 IU; vitamin D3, 1800 IU; vitamin E, 40 mg; vitamin K, 1.8 mg; thiamin, 1.8 mg; riboflavin, 4.4 mg; pyridoxine, 2.8 mg; vitamin B12, 0.02 mg; niacin, 30 mg; folic acid, 2 mg; biotin 0.2 mg;
[5]The mineral premix provided the following quantities of microminerals per kilogram of complete diet: Cu, 7.5 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 0.175 mg as potassium iodate; Mn, 25 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 125 mg as zinc oxide.
[6]Optiphos 2000 (2,000 phytase units per gram), Enzyvia, Sheridan, IN.

were stored in a freezer after collection. At the end of the digestion trial, samples within each animal and diet were thawed, mixed and dried at 105^0C.

Data Gathered

ATTD of P. Each test ingredient was the only P-contributing ingredient in each respective diet. The calculated digestibility value for each diet represented the ATTD of P in each test ingredient. The ATTD (%) of P of each of the test ingredient was calculated as follows (Almeida and Stein, 2010):

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

where ATTD is the apparent total tract digestibility, Pi was the total P intake (g) from d 6 to d 10; and Pf was the total fecal output (g) of P originating from the diet fed from d 6 to d 10.

STTD of P. The STTD of P was calculated using the following equation (Almeida and Stein, 2010):

$$\text{STTD (\%)} = [\text{Pi} - (\text{Pf} - \text{EPL})/\text{Pi}] \times 100$$

where STTD is the standardized total tract digestibility; and Pi and Pf were the total P intake (g) and total fecal output (g), respectively, from the diet given from d 6 to d 10. A basal endogenous loss of 200 mg P per kg DMI was assumed (Stein, 2011). The daily endogenous phosphorus losses (EPL) in pigs fed the P-containing diets were calculated by multiplying the calculated EPL per kilogram of DMI by the daily DMI of each pig.

Chemical Analysis

Dry matter and total phosphorus of the test ingredients, diets, and feces were analyzed in triplicate using the AOAC (2007) procedures. Diets and test ingredients were also analyzed for CP, crude fiber, crude fat, ADF, NDF, ash and calcium using the same procedure.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Version 9.1.3) with pig as the experimental unit. The model included diet, phytase, and diet × phytase as fixed effects and block as the random effect. Least squares means were calculated for each independent variable and means were separated using the PDIFF option. Level of significance and tendencies were set at $P \leq 0.05$ and $P < 0.10$, respectively, for all statistical tests.

Experiment 2. *In vitro* determination of P release from corn, SBM and RBD1.

In vitro procedure developed by Liu *et al*. (1997) was used to estimate phosphorus release of diets used in the experiment 1. The procedure was composed of two enzymatic digestion, the peptic digestion and pancreatic digestion, which basically simulates the environment in the stomach and small intestine of swine, wherein microbial dephosphorylation and mineral absorption occur. For peptic digestion, wherein P digestion in the stomach was simulated, 1-g (±0.001) of finely ground (1.0mm screen) feed sample was mixed with 2mL of a 0.18 N HCl solution containing 1500 U of

pepsin/mL; sealed, vortexed and incubated at 39°C for 75 minutes. The sample was then subjected to pancreatic digestion (simulation of P digestion in the small intestine) wherein 0.65 mL of a 1 M $NaHCO_3$ containing 3.7 mg pancreatin/mL was added and mixed. Slurry was then transferred to dialysis tubing (12000 to 14000 MW cut-off), sealed with closure, and then dialyzed in 100 mL of a 0.05 M pH 6.0 succinate buffer containing 0.1 M NaCl and 0.02% sodium azide at 39°C for 60 to 240 minutes. Finally, phosphorus concentration in succinate buffer was determined using the procedure described by AOAC (1984). Potassium dihydrogen phosphate (KH_2PO_4) was used as standard using the preparation described by AOAC (1984). The digestibility values obtained were correlated with the results of the *in vivo* trials.

The standards, succinate buffers and blank samples were analyzed for absorbance using spectrophotometer. The absorbance of standards and corresponding P concentration were analyzed for regression equation. This equation was used to estimate the P content of the diet (diet P) and blank samples (blank P). Blank was used to correct for the absorbance other than P from the diets. The formula in determining release of P is shown below:

***In vitro* release of P, %** = (P in succinate buffer/total P of the diet)*100

where P inside the succinate buffer was calculated by subtracting the blank P from diet P and multiplying by the dilution factor; total P of the diet was the analyzed P content of each diet on dry matter basis.

Study 2. Performance of Growing Pigs Fed Diets Formulated Based on Standardized Total Tract Digestibility of Phosphorus With and Without Phytase

Experimental Design

A total of 36 barrows (PIC L337 x C24, initial BW = 33.0± 2.7kg) were randomly allotted to four treatment groups following a 2 x 2 factorial arrangement in a randomized complete block design. The same set of animals used in study 1 was also used in this study. Factors involved were inclusion of RBD_1 (0 and 10%) and inclusion of phytase (0 and 500 FTU/kg), with body weight and location as blocking factors. Each treatment diet was replicated 9 times with each pig penned individually. The treatments were as follows: corn-SBM with and without phytase, and corn-SBM-RBD_1 with and without phytase.

Experimental Diets

Four diets were formulated using the STTD of P (Table 3) in corn, SBM and RBD_1 obtained from study 1, experiment 1, wherein two different values of STTD of P per ingredient were used in formulating diets with and without phytase. Two diets were formulated consisting of corn-SBM with and without RBD_1 as basal diets. Two additional diets identical to the basal diets were also formulated with the exception that 500 units of phytase were added. All diets were formulated to contain 0.31% STTD of P. Vitamins and all minerals were included in the diets according to NRC requirements (NRC, 1998). When calculated for available phosphorus content, the experimental diets contained

different available phosphorus values. For corn-SBM with and without phytase, available phosphorus values were 0.20 and 0.28%; for corn-SBM- RBD_1 with and without phytase, 0.12 and 0.22%, respectively.

Digestion Trial

Feed and water were given on *ad libitum* basis. Pigs were fed their respective diet for 28 days. On the 24^{th} day, 0.2% chromic oxide was included in the diet to serve as a marker. Grab sampling of feces was done when fecal matters turned totally green. The samples were then stored in a freezer. After 3 days of collection, the feces were then pooled per animal per diet and prepared for analysis.

Data Gathered

Average daily gain (ADG). Initial and final body weights of each pig were measured. Gain in weight was obtained by subtracting the initial body weight from final body weight of the animal. It was then divided by the number of feeding trial days to determine ADG.

Average daily feed intake (ADFI). Feed intake was calculated by deducting the total amount of feed refused and wasted from the total amount of pre-weighed offered during the whole feeding period. It was then divided by the number of feeding days to obtain ADFI.

Feed efficiency (G: F). Gain in weight was divided by the amount of feed intake during the feeding period.

Table 3. Ingredient composition and nutrient content of grower diets with and without phytase (as-fed basis).

DIET:	CORN-SBM		CORN-SBM- RBD_1	
Ingredient Phytase, FTU/kg[3]:	0	500	0	500
Corn yellow (local)	78.85	79.01	71.46	71.68
Soybean meal, US high	15.70	15.70	13.12	13.12
Rice bran D_1	-	-	10.00	10.00
Coconut oil	3.00	3.00	3.00	3.00
Monocalcium phosphate	0.86	0.50	0.48	-
Limestone	0.77	0.94	1.00	1.23
L-Lysine	0.32	0.32	0.38	0.38
Salt	0.30	0.30	0.30	0.30
L-Threonine	0.08	0.08	0.11	0.11
Mineral Premix[1]	0.08	0.08	0.08	0.08
Vitamin Concentrate[2]	0.02	0.02	0.02	0.02
DL-Methionine	0.01	0.01	0.03	0.03
L-Tryptophan	0.01	0.01	0.02	0.02
Phytase[3]	-	0.025	-	0.025
Total	100.00	100.00	100.00	100.00
Calculated Analysis				
Crude Protein (Nx6.25), %	13.12	13.13	12.64	12.66
Standardized Ileal Digestible Lys: ME, g/Mcal	2.50	2.50	2.52	2.51
Metabolizable Energy, kcal/kg	3315	3316	3297	3300
Standardized Ileal Digestible Amino Acid[4], %				
Lysine	0.83	0.83	0.83	0.83
Methionine	0.24	0.24	0.25	0.25
Methionie+Cystine	0.47	0.47	0.47	0.47
Threonine	0.52	0.52	0.52	0.52
Tryptophan	0.15	0.15	0.15	0.15
Calcium, %	0.66	0.66	0.66	0.66
Total Phosphorus	0.48	0.40	0.54	0.44
Standardized Total Tract Digestible Phosphorus[5]	0.31	0.31	0.31	0.31

[1]The vitamin premix provided the following quantities of vitamins per kilogram of complete diet: Vitamin A, 10, 000 IU; vitamin D3, 1800 IU; vitamin E, 40 mg; vitamin K, 1.8 mg; thiamin, 1.8 mg; riboflavin, 4.4 mg; pyridoxine, 2.8 mg; vitamin B12, 0.02 mg; niacin, 30 mg; folic acid, 2 mg; biotin 0.2 mg;

[2]The mineral premix provided the following quantities of microminerals per kilogram of complete diet: Cu, 7.5 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 0.175 mg as potassium iodate; Mn, 25 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 125 mg as zinc oxide.

[3]Optiphos 2000 (2,000 phytase units per gram), Enzyvia, Sheridan, IN.

[4]SID AA = standardized ileal digestible amino acid

[5]STTD P = standardized total tract digestible P; values computed based on digestibility values from study 1, experiment 1. For monocalcium phosphate, a value of 88.3% was used (NRC, 2012)

ATTD of P. Digestibility values were obtained as discussed in study 1, experiment 1.

Chemical Analysis

Diets and individual ingredients were analyzed for total P, Ca, CP, and ash (AOAC Int., 2007).

Statistical Analysis

Data were analyzed following a factorial experiment (2x2) using the MIXED procedure of SAS (SAS Version 9.1.3), with pig as the experimental unit. The model included diet, phytase, and diet × phytase as fixed effects and block as the random effect. Least squares means were calculated for each independent variable and means were separated using the PDIFF option. Level of significance and tendencies were set at $P \leq 0.05$ and $P < 0.10$, respectively, for all statistical tests.

Economic Analysis

The economic advantage of using phytase and RBD_1 was analyzed. Total feed cost per animal was calculated by multiplying total feed intake per animal with cost of feed per kilogram using the prevailing price of feed ingredients during the conduct of experiment. Feed cost efficiency was then computed by dividing the total feed cost per animal with total gain in weight per animal. Higher feed cost efficiency value means lower profit.

RESULTS AND DISCUSSION

Study 1. Effect of Phytase on the STTD of Phosphorus in Rice Bran

Experiment 1. In vivo procedure to determine the effect of phytase on the STTD of P in corn, SBM and RBD_1

Nutrient composition of corn, SBM, RBD1 and diets

Table 4 shows the nutrient composition of corn, SBM and RBD_1 used in the study. Crude protein, crude fiber, crude fat, and ash content of corn are relatively lower than the published data for local corn (PHILSAN, 2010). Crude protein and crude fat of SBM are slightly lower than PHILSAN values, while the crude fiber and calcium are almost same. The total P values of corn and SBM were close to PHILSAN values while RBD_1 was relatively higher. Crude protein, crude fat, ash and total P of RBD_1 are relatively higher while crude fiber and calcium are slightly lower than PHILSAN values. This shows the variation in nutrient content of each raw material which consequently affects nutrient composition of a complete diet.

Table 4. Nutrient composition of corn, SBM, and RBD_1.

	CORN		SBM		RBD_1	
Nutrient, %	PHILSAN (2010)	Analyzed	PHILSAN (2010)	Analyzed	PHILSAN (2010)	Analyzed
Moisture	10.71	8.47	9.28	8.68	8.60	10.12
Ash	1.42	1.39	6.60	6.73	6.89	7.05
Crude Protein*	8.05	7.60	47.65	45.41	12.14	12.54
Crude Fiber	2.44	2.25	3.43	3.43	5.27	4.13
Crude Fat	3.94	3.14	1.23	0.90	13.79	14.05
Nitrogen Free Extracts		77.15		34.85		52.11
Calcium	0.17	0.20	0.47	0.50	0.15	0.10
Total Phosphorus	0.26	0.23	0.71	0.74	1.48	1.78

*Analyzed using Kjeldahl method

Nutrient composition of diets (as-fed basis) is shown in Table 5. Each diet contained different amount of crude protein, crude fiber, crude fat, calcium and phosphorus, depending on the raw material used as the sole source of P.

RBD_1 diets had the highest amount of total phosphorus and crude fat among the diets. Addition of phytase did not affect the nutrient composition of the diets since the liberation of nutrients using the enzyme will only take place inside the body of the animal wherein the conditions for the enzyme to work are satisfied. This includes proper pH level (6.0) and temperature (37°C).

Table 5. Nutrient composition of semi-purified diets (as-fed basis).

NUTRIENT	CORN		SBM		RBD_1	
Phytase, FTU/kg[1]	0	500	0	500	0	500
Dry Matter, %	90.28	89.98	92.39	90.71	92.24	92.15
Crude Protein, %	7.52	7.48	17.68	17.63	6.66	6.95
Crude Fiber	1.70	4.51	2.29	1.58	2.28	2.65
Crude Fat	4.38	4.11	1.47	1.08	7.53	8.26
Ash, %	2.60	2.57	4.46	3.54	4.69	4.95
Nitrogen Free Extracts, %	74.08	71.31	66.59	66.88	71.08	69.33
Calcium, %	0.59	0.50	0.50	0.49	0.50	0.60
Total Phosphorus, %	0.27	0.25	0.23	0.35	0.77	0.92

[1]FTU = phytase unit

STTD of phosphorus of corn, SBM and RBD_1

Table 6 shows the effect of phytase on P balance, ATTD and STTD of P in semi-purified corn/SBM/ RBD_1-based diets.

For animals fed semi-purified corn-based diets, addition of phytase reduced phosphorus concentration in the feces and daily phosphorus output from 2.10 to 1.29% ($P<0.01$) and 1.07 to 0.71g ($P<0.05$), respectively. This means the enzyme was able to liberate significant amount of phosphate from the phytate of corn in the stomach and small intestine.

Consequently, addition of phytase increased ($P<0.005$) the ATTD and STTD of P from 47 to 66% and 53.38 to 73%, respectively. The digestibility values for corn without phytase was relatively higher than the values observed by Almeida and Stein in 2010 (19.90% ATTD of P and 26.40% STTD of P) when given to pigs with initial BW of

Table 6. Effect of phytase on P-balance and digestibility of phosphorus in semi-purified corn, SBM and RBD_1-based diets. [1]

	PHYTASE, FTU/kg[2]			
INGREDIENT	0	500	SEM	P-value
CORN				
Feed intake, g/d	680	734	51	0.478
Dry Matter,%	90.28	89.98		
P intake, g/d	2.03	2.04	0.15	0.982
Fecal output, g/d	53.36	62.27	9.02	0.501
P in feces, %	2.1	1.29	0.17	0.008
P output, g/d	1.07	0.71	0.09	0.017
ATTD of P, %	47	66	4	0.005
Basal EPL[3], mg/d	136	147	10	0.478
STTD of P[4], %	53	73	4	0.004
SBM				
Feed intake, g/d	746	670	21	0.03
Dry Matter,%	92.39	90.71		
P intake, g/d	1.86	2.59	0.07	<.0001
Fecal output, g/d	26.8	27.59	3.3	0.869
P in feces, %	4.21	2.99	0.27	0.009
P output, g/d	1.16	0.83	0.15	0.16
ATTD of P, %	38	68	8	0.018
Basal EPL[3], mg/d	149	134	4	0.03
STTD of P[4], %	46	73	8	0.035
RBD_1				
Feed intake, g/d	796	796	47	0.994
Dry Matter,%	92.55	92.15		
P intake, g/d	6.63	7.95	0.42	0.049
Fecal output, g/d	58.4	61.44	5.3	0.694
P in feces, %	5.96	4.89	0.21	0.005
P output, g/d	3.47	3	0.31	0.306
ATTD of P, %	47	62	4	0.012
Basal EPL[3], mg/d	159	159	9	0.994
STTD of P[4], %	50	64	4	0.015

[1]Data are means of 6 observations per diet.

[2]FTU = phytase units.

[3]EPL = endogenous P loss. This value is equivalent to 200mg/kg DMI (NRC, 2012). The daily EPL was computed by multiplying the EPL (mg/kg DMI) by the daily DMI of each diet.

[4]STTD was calculated by subtracting basal EPL from ATTD.

13.5 ± 3.9 kg and Bohlke *et al*. in 2005 (28.8% ATTD of P) when fed to growing barrows (initial BW = 29.3 ± 1 kg). The digestibility of P in corn was higher but within the range of published NRC (2012) values, ranging from 12 to 48% ATTD of P and 27 to 41% STTD of P. This indicates wide variation in phytate phosphorus in corn which can be associated to variable response observed when phytase was supplemented in corn-SBM based diets.

For SBM, lower feed intake ($P<0.05$) was observed in animals given diet with phytase (670.13g) than without the enzyme (745.78g). At the start of the experiment, two pigs given the SBM with phytase diet were already sick, thus affecting the feed intake. In effect, this resulted to higher ($P<0.05$) basal EPL in animals given this diet (149 vs. 134mg).

Phosphorus concentration in feces was reduced ($P<0.01$) upon supplementation of phytase in SBM diet from 4.2 to 2.99%. Accordingly, ATTD and STTD of P in SBM increased ($P<0.05$) from 38 to 68% and from 46 to 75%, respectively, which were almost the same with the values obtained in the previous studies on P digestibility of raw materials conducted at the University of Illinois-Urbana Champagne (39 ± 6.24% ATTD of P and 48±7.62% STTD of P) (NRC, 2012). This clearly validates the procedure and results obtained in this experiment. Almost the same increase ($P < 0.01$) in ATTD and STTD of P was observed by Rojas and Stein (2012) when they supplemented the same phytase on conventional SBM (from 41.6 to 66.2% and from 46.1 to 71.4%, respectively). The ATTD and STTD of P values were higher than the RBV of P, which accounts only 29.6% of the total P in SBM were bioavailable for the animals based on

PHILSAN values (PHILSAN, 2010). This indicates that the RBV evaluation system underestimates the bioavailability of P in SBM, resulting to excess P in the diets and greater risk for the environment.

As observed in corn and SBM, addition of phytase decreased ($P = 0.005$) the concentration of P (from 5.96 to 4.89%) in the feces of the animals fed with semi-purified RBD_1-based diet. Thus, ATTD and STTD of P were increased ($P<0.05$) from 47 to 62% and from 50 to 64%, respectively. The values obtained were more than three times higher than the published values (12-14% ATTD of P and 22-24% STTD of P according to NRC, 2012; and RBV of P value of 15% according to PHILSAN, 2010). This shows that P bioavailability in RBD_1 had been underestimated and hence, inefficiently utilized as a source of P. In effect, diets formulated with RBD_1 using the published values were more expensive as they contained P more than the requirement of the animal.

Experiment 2. *In vitro* determination of P release in corn, SBM and RBD_1

Table 7 shows the i*n vitro* release of phosphorus in corn, SBM and RBD_1 with and without phytase. The amount of phosphorus in the succinate buffer was equated as the TTTD P, as succinate buffer contained the P released during peptic and pancreatic digestion, simulating the P digestion and absorption in the stomach and small intestine and without endogenous secretion of P or EPL.

Using corn as a substrate, release of P was improved from 5.40% to 38.5%, with 7.13 times improvement upon supplementation of phytase. Release of P from SBM was

Table 7. *In vitro* release of phosphorus in corn, SBM and RBD_1 with and without phytase.[1]

INGREDIENT	IN VITRO	
Phytase, FTU/kg[2]	0	500
Corn		
Total phosphorus (DM), mg	2.44	2.45
Phosphorus in succinate buffer, mg	0.13	1.07
In vitro P release, %	5.40	38.50
SBM		
Total phosphorus (DM), mg	2.12	3.18
Phosphorus in succinate buffer, mg	0.12	0.85
In vitro P release, %	5.80	26.70
RBD_1		
Total phosphorus (DM), mg	7.13	8.48
Phosphorus in succinate buffer, mg	0.21	1.61
In vitro P release, %	2.90	19.00

[1]Data are means of 6 observations per treatment.

[2]FTU = phytase units.

increased from 5.80%, to 26.70% with phytase supplementation, which is 4.6 times improvement.

For RBD_1, release of P was improved by 6.55 times from 2.9 to 19.0% upon addition of phytase. RBD_1 had the lowest amount of phosphorus liberated among the ingredients, which could be attributed to the lower percent phosphorus bound in the form of phytate in the said ingredient. The result shows that addition of phytase improved the digestibility of phosphorus in each raw material.

Table 8 shows the correlation of STTD of P of corn, SBM and RBD_1 using *in vivo* and release of P using *in vitro* procedures. The *in vitro* release of P was highly correlated ($r=0.94$, $P=0.005$) with the *in vivo* P digestibility. This means the *in vitro* procedure was able to detect improvement in the digestibility of phosphorus when phytase was supplemented, as observed in the *in vivo* procedure. This validates the observation in the *in vivo* study.

Table 8. Correlation of P release of corn, SBM and rice bran D_1 using *in vivo* and *in vitro* procedure. [1]

INGREDIENT	DIET	
Phytase, FTU/kg[2]	0	500
Corn		
In vivo STTD of P, %	53.4	73.4
In vitro P release, %	5.4	38.5
SBM		
In vivo STTD of P, %	46.1	73.1
In vitro P release, %	5.8	26.7
RBD_1		
In vivo STTD of P, %	49.8	64.4
In vitro P release, %	2.9	19
Correlation Value	r = 0.94	
P-value	0.0052	

[1]Data are means of 6 observations per treatment.

[2]FTU = phytase units.

The percent improvement in the P release was higher in the *in vitro* procedure than the STTD P values obtained using *in vivo* procedure when phytase was supplemented in the test ingredients. For corn, *in vivo* digestibility of P improved by 37.5%, while the *in vitro* P release increased by more than 7 times. Likewise, *in vivo* digestibility of P in SBM increased by 58.6% and *in vitro* P release improved by more than 5 times. For RBD_1, *in vivo* digestibility of P improved by 29.3%, while *in vitro* P release increased by more than 6 times. The variation between both procedures can be attributed to differences in the actual digestion conditions (pH, temperature, digestion time, among others), thus affecting the activity of enzymes and consequently the release of P.

The result in this experiment agrees with the study conducted by Schlegel *et al.* (2012) on *in vitro* evaluation of digestible phosphorus in corn, wheat, barley, soybean meal, potato protein concentrate and rapeseed cake. The *in vitro* P release values were slightly lower than the *in vivo* digestible P values (15.9 vs. 21% for corn; 32.6 vs. 36.3% for barley; 48.1 vs. 48.6% for wheat; 36.5 vs. 40.4% for potato protein; 22.8 vs. 33.2% for rapeseed cake; and 29.8 vs. 39.3% for soybean cake) but were highly correlated. The *in vivo* digestible P values for corn and SBM in this experiment were higher than that of Schlegel *et al.* (2012), but lower in *in vitro* digestible P values. This could be attributed to differences in amount of samples analyzed; additional procedure before pepsin digestion (soaking the sample); and pH level and length of time the samples were subjected to peptic and pancreatic digestion.

Figure 3 shows the regression analysis of *in vivo* STTD of P and in vitro P release values. Low P value (P=0.005) of regression analysis indicates that P release values using *in vitro* procedure were significantly related to changes in the *in vivo* STTD of P. The regression equation (y = 0.7869x + 47.142) represents the mean change in the *in vivo* STTD of P for one unit of change in the *in vitro* P release while holding other predictors in the linear regression model constant. This means for every additional 1% in *in vitro* P release, the *in vivo* STTD of P is expected to increase by an average of 0.7869%. Regression analysis (R^2 = 0.8851) indicates that the linear regression equation explains 88% variability of the response data (*in vivo* STTD of P) around its mean. In general, the higher the R^2, the better the equation in estimating *in vivo* STTD of P.

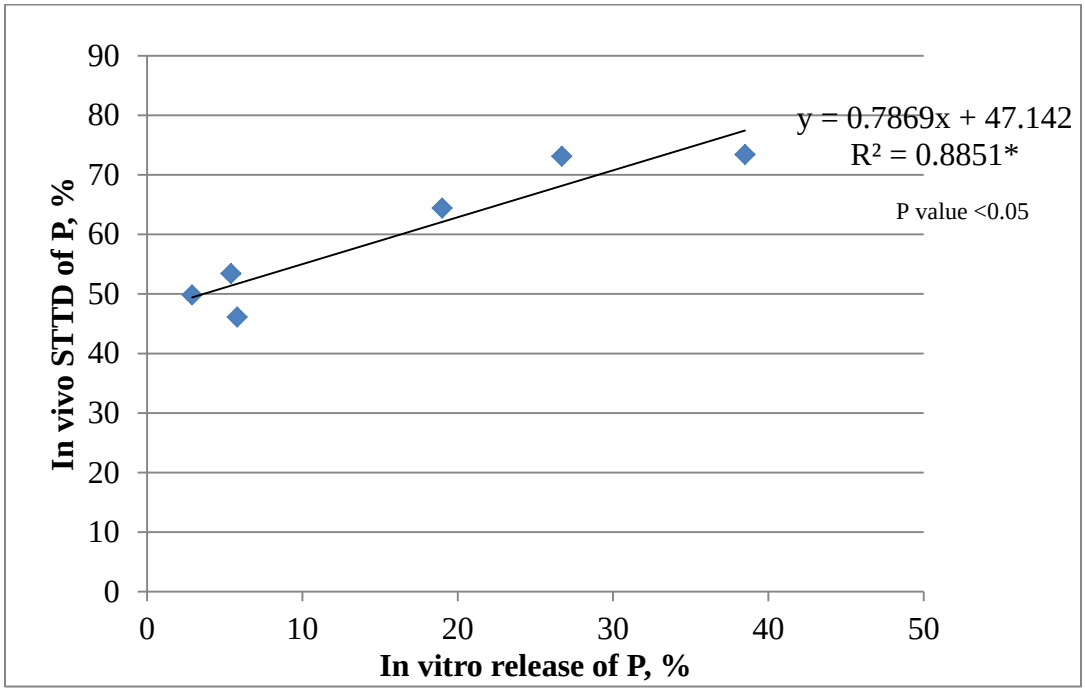

Figure 3. Regression analysis of *in vivo* STTD of P and *in vitro* release of P.

Study 2. Performance of Growing Pigs Fed Diets Formulated Based on Standardized Total Tract Digestibility of Phosphorus With and Without Phytase

This study is basically a validation of the digestibility coefficients obtained in study 1, experiment 1, wherein the animals were expected to have the same performance given diets formulated to contain the same amount of nutrients with coefficient of STTD of P of ingredients obtained in study 1, experiment 1.

It is a common practice that inorganic P is being supplemented in monogastric diets because of the poor digestibility of P in corn and SBM. With the use of the values of STTD of P and phytase, supplementation of inorganic P, MCP for this experiment, was reduced. Likewise, use of RBD_1 as ingredient and phytase supplementation could totally eliminate the use of inorganic P. The same was observed by Almeida (2010), wherein DCP was not supplemented in the diet upon supplementation of phytase and DDGS. The idea was that the animal will have the same performance given diets were formulated to provide the same amount of nutrients particularly of STTD of P regardless of the total P level of the diets.

Nutrient Composition of Diets

Table 9 shows the nutrient composition of the diets (as-fed basis). The release of P with the use of enzyme was already accounted when different STTD P values per ingredient was used in formulating the diets with and without phytase supplementation, resulting to low or non-inclusion of inorganic P.

Table 9. Nutrient composition of diets (as-fed basis).

NUTRIENT	CORN-SBM[1]		CORN-SBM- RBD_1[2]	
Phytase, FTU/kg[3]	0	500	0	500
Dry matter, %	88.31	88.5	88.37	88.29
Crude protein, %	13.84	13.65	13.67	13.61
Crude fiber, %	1.82	2.16	2.26	2.53
Crude fat, %	4.52	4.68	5.64	6.47
Ash, %	3.56	3.16	3.83	3.48
Nitrogen-free extracts, %[4]	64.57	64.85	62.97	62.20
Calcium, %	0.43	0.35	0.40	0.37
Total P, %	0.50	0.41	0.56	0.43

[1]SBM = soybean meal

[2] RBD_1 = rice bran D1

[3]FTU = phytase unit

[4]NFE = nitrogen-free extract

Corn-SBM-RBD_1 based diets had higher crude fiber (2.16 and 2.53% vs. 1.82 and 2.26%) and higher crude fat (4.68 and 6.47% vs. 4.52 and 5.64%) than corn-SBM diets due to high fiber and fat composition of RBD_1. Crude protein (13.69% on the average) and dry matter content (average of 88.37%) were almost the same in all diets.

The diets formulated based on the STTD of P contained lower total phosphorus (0.41 and 0.42% vs. 0.50 and 0.56%) as compared with the diets without the enzyme. This is due to reduction or complete elimination of MCP when RBD_1 and/or phytase were added in the diet.

Table 10 shows the summary of the effect of RBD_1 and phytase supplementation on growth performance of growing pigs. There was neither interaction effect (P>0.05) nor main effect (P>0.10) of RBD_1 and phytase supplementation in all production parameters measured. However, there was a significant interaction effect of phytase and RBD_1 on the ATTD of P. This was expected with the higher phytate substrate of RBD_1 and improved digestibility of P in the diets supplemented with enzyme.

Table 10. Growth performance of growing pigs fed with diets supplemented with RBD_1 and phytase. [1]

Diet	PHYTASE, FTU/kg[2]	INITIAL BW, kg	FINAL BW, kg	ADG, kg	ADFI, kg	G:F	ATTD of P, %
Corn-SBM	0	32.67	58.8	0.93	2.22	0.42	31.00
	500	33.37	61.95	1.02	2.22	0.46	66.00
Corn-SBM-RBD_1[3]	0	32.86	60.08	0.97	2.26	0.43	41.00
	500	33.06	61.2	1.00	2.27	0.44	54.00
	SEM	0.94	1.59	0.05	0.03	0.02	5.00
P-value	RBD_1	0.95	0.867	0.823	0.104	0.944	0.876
	Phytase	0.632	0.188	0.254	0.893	0.26	0.0001
	RBD_1 x Phytase	0.793	0.525	0.598	0.775	0.504	0.045

[1]Data are means of 9 observations per treatment

[2]FTU = phytase units

[3]Diets contained 10% RBD_1

Body Weight

Initial body weight of growing pigs ranged from 32.67 to 33.37 kg which indicates that experimental animals were uniform in body weight at the start of the experiment.

After 30 days of feeding, the average final body weight of growing pigs fed with corn-SBM diets with and without phytase were 61.95 and 58.8 kg, respectively; whereas those fed with corn-SBM- RBD_1 diets with and without phytase had an average of 61.2 and 60.08 kg, respectively. Consequently, the ADG of the animals did not differ among treatments (ranging from 0.93 to 1.02 kg). This indicates that the phytase was able to release significant amount of phosphorus from the phytic acid and the RBD_1 can partially replace other ingredients without negative effect on the performance of animals.

Feed Consumption

The average daily feed intake (2.24kg; ranging from 2.22 to 2.27 kg) was the same across treatment. There was no observed interaction effect of RBD1 and enzyme supplementation. Likewise, the two factors did not elicit significant mean effects on feed consumption. Addition of RBD_1 and phytase did not negatively affect the palatability of diets.

Feed Efficiency

Since there was no significant difference observed in the weight gain and feed consumption of animals given the treatment diets, there was also no difference in the feed efficiency in this experiment.

ATTD of P

There was an interaction effect ($P< 0.05$) of RBD_1 and phytase on the ATTD of P. The corn-SBM diets with and without phytase had 31 and 66% ATTD of P while corn-SBM- RBD_1 diets with and without phytase had 41 and 54% ATTD of P. This means the supplementation of RBD_1 and phytase improved the digestibility of phosphorus. The improvement in the ATTD of P was not manifested in the performance of the animals because the phosphorus requirement was already satisfied and any increase in phosphorus level will not elicit improvement in the performance.

Economic Analysis

Table 11 shows the economic analysis of the diets. Results show that supplementation of phytase, rice bran or combination of phytase and rice bran improved feed efficiency by 4.47, 2.52 and 3.84 PhP/kg, respectively. Profit was increased when phytase, rice bran or combination of phytase and rice bran were added in swine diets.

Table 11. Economic analysis of diets.

DIET	CORN-SBM		CORN-SBM- RBD_1	
Item Phytase, FTU/kg	0	500	0	500
Total wt gain/pig, kg	26.12	28.58	27.22	28.13
Total feed intake/pig, kg	62.17	62.04	63.27	63.61
Feed cost/kg, PhP	21.83	21.87	21.27	21.28
Total feed cost/pig, PhP	1356.82	1356.97	1345.59	1353.39
Feed cost efficiency,[1] PhP/kg	51.95	47.48	49.43	48.11
Feed cost efficiency vs. corn-SBM without phytase and RBD_1, PhP/kg		(-4.47)	(-2.52)	(-3.84)

[1]Feed cost efficiency = $\frac{\text{total feed cost/pig}}{\text{total weight gain/pig}}$

SUMMARY AND CONCLUSION

Two studies were conducted to determine the standardized total tract digestible (STTD) phosphorus (P) in rice bran, with corn and SBM as reference ingredients. The first study was composed of two experiments, the *in vivo* determination of STTD of P and the use of *in vitro* procedure in estimating release of P. The objectives of the first study were to determine the STTD P values in the ingredients with and without phytase when fed to growing pigs and estimate P release using *in vitro* procedure. The second study aimed to determine the effect of diets formulated with the same level of STTD P on the growth performance of growing pigs regardless of total and available P level using the coefficients in the first experiment of study 1; and evaluate the effect of phytase and RBD_1 on feed cost efficiency.

In study 1, 36 barrows (initial BW = 22.3 ± 1.4kg) were randomly allotted to six semi-purified diets with each ingredient as the sole source of P with and without phytase. Each pig was housed in metabolism cages that allowed for total collection of feces. Basal endogenous P losses (EPL) were estimated to be 200mg/kg DMI. In study 2, the same set of animals (initial BW: 33 ± 2.7kg) were randomly allotted to four corn-SBM based diets following a 2 x 2 factorial in a randomized complete block design. Factors were phytase (0 and 500FTU/kg; Optiphos 2000, Enzyvia, Sheridan, IN) and rice bran (0 and 10%), with initial body weight as a blocking factor. All diets were formulated to contain 0.31% STTD P and fed to growing pigs for 28 d.

Results were as follows:

Study 1. Effect of Phytase on the STTD of Phosphorus in Rice Bran

1. Phytase supplementation improved ($P<0.05$) the ATTD of P in corn from 47 to 66%, in SBM from 38 to 68%, and in rice bran from 47 to 62%.
2. Addition of phytase increased ($P<0.05$) the STTD of P in corn from 53.38 to 73.35%, in SBM from 46.12 to 73.05%, and in rice bran from 49.76 to 64.43%.
3. The in vitro release of P was highly correlated ($r = 0.94$) with the in vivo P digestibility. Linear regression equation showed that for every additional 1% in *in vitro* P release, the *in vivo* STTD of P is expected to increase by an average of 0.7869%.

Study 2. Performance of Growing Pigs Fed Diets Formulated Based on Standardized Total Tract Digestibility of Phosphorus With and Without Phytase

1. There was no ($P > 0.05$) phytase × rice bran interaction in all the growth parameters measured. There was also no ($P > 0.10$) difference in ADG, ADFI, G:F and final BW between pigs diets with and without phytase and diets with 0 or 10% rice bran.
2. Feed cost efficiency was better when phytase and RBD_1 or combination of phytase and RBD_1 were included in growing pig diet.

Based on the results, the phytase was able to improve the STTD of P in corn, SBM and rice bran, and the *in vitro* procedure by Liu *et al*. (1997) can be used to estimate the *in vivo* phosphorus release in corn, SBM and RBD_1 fed to growing pigs. Also, growing pig diets can be formulated based on STTD of P. Supplementation of inorganic P can be reduced or eliminated without reducing animal performance when using phytase, rice bran or combination of phytase and rice bran in the diet. Thus, more profit may be generated when phytase and RBD_1 were included in pig diets.

RECOMMENDATIONS

Based on the results of this study, the author highly recommends the following:

1. Use STTD of P values instead of available P of ingredients when formulating diets to improve efficiency and profitability.
2. Due to differences in feed milling process, variation of STTD of P among different rice bran sources must be evaluated.
3. STTD of P of other alternative ingredients such as copra and cassava meal must be determined to complete the database for STTD P values.
4. For the *in vitro* procedure, more samples and additional levels of phytase are recommended to further explore relationships with *in vivo* results.

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APPENDIX TABLES

Appendix Table 1. Least square mean of feed intake of pigs fed semi-purified corn-based diets (g/day, DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	0.54	0.4782
Method = LSQ (P<0.05)				
Obs	Treatment	Estimate	Standard Error	Letter Group
1	Corn (0)	679.92	51.4782	A
2	Corn (500)	733.56	51.4782	A

The Mixed Procedure
Type 3 Tests of Fixed Effects

Appendix Table 2. Least square mean of P intake of pigs fed semi-purified corn-based diets (g/day, DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	0.00	0.9824
Method = LSQ (P<0.05)				
Obs	Treatment	Estimate	Standard Error	Letter Group
1	Corn (0)	2.0334	0.1461	A
2	Corn (500)	2.0381	0.1461	A

Appendix Table 3. Least square mean of fecal output (g/day) of pigs fed semi-purified corn-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	0.49	0.5012

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	Corn (0)	53.3616	9.0229	A
2	Corn (500)	62.2664	9.0229	A

Appendix Table 4. Least square mean of P in feces (%) of pigs fed semi-purified corn-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	10.82	0.0082

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	Corn (0)	2.0994	0.1742	A
2	Corn (500)	1.2892	0.1742	B

Appendix Table 5. Least square mean of P output (g/day) of pigs fed semi-purified corn-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	8.25	0.0166
		Method = LSQ (P<0.05)		
Obs	Treatment	Estimate	Standard Error	Letter Group
1	Corn (0)	1.0743	0.08964	A
2	Corn (500)	0.7101	0.08964	B

Appendix Table 6. Least square mean of ATTD P of pigs fed semi-purified corn-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	12.64	0.0052
		Method = LSQ (P<0.05)		
Obs	Treatment	Estimate	Standard Error	Letter Group
1	Corn (0)	46.5223	3.8835	B
2	Corn (500)	66.0464	3.8835	A

Appendix Table 7. Least square mean of basal EPL of pigs fed semi-purified corn-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	0.54	0.4782

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	Corn (0)	135.98	10.2956	A
2	Corn (500)	146.71	10.2956	A

Appendix Table 8. Least square mean of STTD P of pigs fed semi-purified corn-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	13.63	0.0042

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	Corn (0)	53.3821	3.8248	B
2	Corn (500)	73.3546	3.8248	A

Appendix Table 9. Least square mean of feed intake (g/day) of pigs fed semi-purified SBM-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	6.40	0.0298

Method = LSQ (*P*<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	SBM (0)	745.78	21.1371	A
2	SBM (500)	670.13	21.1371	B

Appendix Table 10. Least square mean of P intake (g/day) of pigs fed semi-purified SBM-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	52.58	<.0001

Method = LSQ (*P*<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	SBM (0)	1.8566	0.07110	B
2	SBM (500)	2.5857	0.07110	A

Appendix Table 11. Least square mean of fecal output (g/day) of pigs fed semi-purified SBM-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	0.03	0.8687

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	SBM (0)	26.7994	3.2961	A
2	SBM (500)	27.5899	3.2961	A

Appendix Table 12. Least square mean of P in feces (%) of pigs fed semi-purified SBM-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	10.26	0.0094

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	SBM (0)	4.2059	0.2679	A
2	SBM (500)	2.9922	0.2679	B

Appendix Table 13. Least square mean of P output (g/day) of pigs fed semi-purified SBM-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	2.31	0.1598

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	SBM (0)	1.1568	0.1542	A
2	SBM (500)	0.8256	0.1542	A

Appendix Table 14. Least square mean of ATTD P of pigs fed semi-purified SBM-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	7.99	0.0180

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	SBM (0)	37.8370	7.5122	B
2	SBM (500)	67.8660	7.5122	A

Appendix Table 15. Least square mean of basal EPL of pigs fed semi-purified SBM-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	6.40	0.0298

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	SBM (0)	149.16	4.2274	A
2	SBM (500)	134.03	4.2274	B

Appendix Table 16. Least square mean of STTD P of pigs fed semi-purified SBM-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	5.95	0.0348

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	SBM (0)	46.1185	7.8042	B
2	SBM (500)	73.0495	7.8042	A

Appendix Table 17. Least square mean of feed intake (g/day) of pigs fed semi-purified RBD1-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	0.00	0.9936

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	SBM (0)	796.38	47.0894	A
2	SBM (500)	795.83	47.0894	A

Appendix Table 18. Least square mean of P intake (g/day) of pigs fed semi-purified RBD1-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	5.01	0.0492

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	SBM (0)	6.6257	0.4170	B
2	SBM (500)	7.9453	0.4170	A

Appendix Table 19. Least square mean of fecal output (g/day) of pigs fed semi-purified RBD1-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	0.16	0.6939

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	SBM (0)	58.3989	5.3044	A
2	SBM (500)	61.4378	5.3044	A

Appendix Table 20. Least square mean of P in feces (%) of pigs fed semi-purified RBD1-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	12.90	0.0049

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	SBM (0)	5.9573	0.2106	A
2	SBM (500)	4.8876	0.2106	B

Appendix Table 21. Least square mean of P output (g/day) of pigs fed semi-purified RBD1-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	1.17	0.3058

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	SBM (0)	3.4699	0.3057	A
2	SBM (500)	3.0033	0.3057	A

Appendix Table 22. Least square mean of ATTD P of pigs fed semi-purified RBD1-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	9.54	0.0115

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	SBM (0)	47.1025	3.5091	B
2	SBM (500)	62.4266	3.5091	A

Appendix Table 23. Least square mean of basal EPL of pigs fed semi-purified RBD1-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	0.00	0.9936

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	SBM (0)	159.28	9.4179	A
2	SBM (500)	159.17	9.4179	A

Appendix Table 24. Least square mean of STTD P of pigs fed semi-purified RBD1-based diets (DM basis) with and without phytase on P digestibility study (Study 1, experiment 1)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	1	10	8.66	0.0147

Method = LSQ (P<0.05)

Obs	Treatment	Estimate	Standard Error	Letter Group
1	SBM (0)	49.7581	3.5248	B
2	SBM (500)	64.4299	3.5248	A

Appendix Table 25. Analysis of variation for regression analysis of in vitro and in vivo results (Study 1, experiment 2)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	629.62464	629.62464	30.82	0.0052
Error	4	81.70869	20.42717		
Corrected Total	5	711.33333			

Appendix Table 26. Least square mean of initial body weight of barrows fed corn-SBM based diets with and without RBD1 and phytase (Study 2)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	3	32	0.10	0.9578

Method = LSQ (P<0.05)

Obs Group	Estimate	Standard Error	DF	t Value	Pr > |t|
Corn-SBM (0)	32.6711	0.9359	32	34.91	<.0001
Corn-SBM (500)	33.3711	0.9359	32	35.66	<.0001
Corn-SBM- RBD_1 (0)	32.8600	0.9359	32	35.11	<.0001
Corn-SBM- RBD_1 (500)	33.0644	0.9359	32	35.33	<.0001

Appendix Table 27. Least square mean of final body weight of barrows fed corn-SBM based diets with and without RBD1 and phytase (Study 2)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	3	32	0.75	0.5329

Method = LSQ (P<0.05)

Obs Group	Estimate	Standard Error	DF	t Value	Pr > |t|
Corn-SBM (0)	58.7956	1.5852	32	37.09	<.0001
Corn-SBM (500)	61.9467	1.5852	32	39.08	<.0001
Corn-SBM- RBD_1 (0)	60.0822	1.5852	32	37.90	<.0001
Corn-SBM- RBD_1 (500)	61.1956	1.5852	32	38.60	<.0001

Appendix Table 28. Least square mean of ADG of barrows fed corn-SBM based diets with and without RBD1 and phytase (Study 2)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	3	32	0.56	0.6457

Method = LSQ (P<0.05)

Obs Group	Estimate	Standard Error	DF	t Value	Pr > |t|
Corn-SBM (0)	0.9330	0.05165	32	18.06	<.0001
Corn-SBM (500)	1.0206	0.05165	32	19.76	<.0001
Corn-SBM- RBD_1 (0)	0.9723	0.05165	32	18.83	<.0001
Corn-SBM- RBD_1 (500)	1.0047	0.05165	32	19.45	<.0001

Appendix Table 29. Least square mean of ADFI of barrows fed corn-SBM based diets with and without RBD1 and phytase (Study 2)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	3	32	0.58	0.6304

Method = LSQ (P<0.05)

Obs Group	Estimate	Standard Error	DF	t Value	Pr > |t|
Corn-SBM (0)	2.3274	0.02881	32	80.77	<.0001
Corn-SBM (500)	2.3091	0.02881	32	80.14	<.0001
Corn-SBM- RBD_1 (0)	2.3470	0.02881	32	81.46	<.0001
Corn-SBM- RBD_1 (500)	2.3591	0.02881	32	81.88	<.0001

Appendix Table 30. Least square mean of G:F of barrows fed corn-SBM based diets with and without RBD1 and phytase (Study 2)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	Pr > F
Treatment	3	32	0.59	0.6246

Method = LSQ (P<0.05)

Obs Group	Estimate	Standard Error	DF	t Value	Pr > |t|
Corn-SBM (0)	0.4189	0.02152	32	19.46	<.0001
Corn-SBM (500)	0.4581	0.02152	32	21.29	<.0001
Corn-SBM- RBD_1 (0)	0.4319	0.02152	32	20.07	<.0001
Corn-SBM- RBD_1 (500)	0.4420	0.02152	32	20.54	<.0001

Appendix Table 31. Least square mean of ATTD P of barrows fed corn-SBM based diets with and without RBD1 and phytase (Study 2)

The Mixed Procedure
Type 3 Tests of Fixed Effects

EFFECT	NUM DF	DEN DF	F VALUE	PR > F
Treatment	3	30	8.25	0.0004
Phytase	1	30	19.20	0.0001

Method = LSQ (P<0.05)

Obs Group	Estimate	Standard Error	Letter Group
Corn-SBM (0)	30.6190	5.2929	C
Corn-SBM (500)	65.9378	5.2929	A
Corn-SBM- RBD_1 (0)	41.1774	5.6140	BC
Corn-SBM- RBD_1 (500)	53.6681	5.6140	AB