ABSTRACT

MCAULEY, COOPER THOMAS. Bioavailability of Phosphorus in Turkey Litter Ash and Dried Swine Lagoon Sludge Fed to Growing Pigs (Under the direction of Dr. Eric van Heugten).

An experiment was performed to evaluate two recycled animal waste byproducts as a potential alternative phosphorus (P) source for swine. Fifty-six individually housed pigs (BW of 34.92±0.29 kg) were blocked by initial BW and randomly allocated within block to 1 of 7 dietary treatments. Analyzed concentrations (as-is basis) of Ca, P, and Na were 8.31, 3.98, and 0.70, respectively for turkey litter ash (TLA) and 5.81, 4.31, and 0.12 for dried swine lagoon sludge (SLS). Diets consisted of a negative control basal (NC; 0.50% Ca and 0.12% available P, representing 40% of requirement), NC plus 0.075% P from either monosodium phosphate (MSP), TLA, or SLS, and NC plus 0.150% P from either MSP, TLA, or SLS. Diets were balanced using MSP, calcium carbonate, salt and fine sand to maintain all other dietary ingredients at equal levels. All nutrient requirements were met in the basal diet except for Ca, P, and Na. A constant Ca:P ratio of 1.40 was maintained. Pigs were limit-fed individually at 3×197 kcal ME/kg $BW^{0.60}$ based on the mean BW for each block. Overall ADG linearly increased (P=0.022) with increasing P from MSP (761, 814, and 839 g/d), tended to increase (P = 0.072) with P from TLA (761, 786, and 810 g/d), and was not impacted (P = 0.164) by P from SLS (761, 797, and 807 g/d). Serum P analyzed using a serum chemistry panel linearly increased (P = 0.007) with increasing MSP (8.76, 9.08, and 10.00 mg/dL), but not with TLA (P = 0.232; 8.76, 8.55, and 9.28 mg/dL) or SLS (P = 0.247; 8.76, 9.18, and 9.25 mg/dL). Serum P analyzed by wet chemistry increased linearly in pigs supplemented with MSP (P = 0.006; 16.11, 16.53, and 18.37 mg/dL), and tended to increase with SLS (P = 0.094; 16.11, 16.76, and 17.11 Mg/dL), but no difference was detected with TLA (P = 0.41; 16.11, 16.72, 16.73 Mg/dL). Bone ash weight increased in pigs supplemented with MSP (P = 0.022; 3.62, 3.85, and 4.04 g), TLA (P = 0.005;

3.62, 3.86, and 4.05 g) and SLS (P = 0.015; 3.62, 3.93, and 4.05 g). Bone ash weight by percent tended to linearly increase for pigs fed MSP (P = 0.082; 40.28, 40.98, 43.62%), but no difference was detected in pigs supplemented with TLA or MSP. Total bone P increased linearly in pigs supplemented with MSP (P = 0.017; 0.16, 0.17, and 0.18 %), SLS (P = 0.028; 0.16, 0.17, and 0.19 %) and tended to increase with TLA (P = 0.057; 0.16, 0.18, and 0.18 %). Peak bone breaking force linearly increased with the addition of MSP (P=0.008; 65.53, 72.01, and 84.1 kg) and TLA (P = 0.015; 65.53, 64.95, and 81.06 kg), and tended to increase with SLS (P = 0.077; 65.53, 71.49, 77.18). Bioavailability of P with bone strength as criteria was 73.75% for TLA and 67.1% for SLS. Apparent total tract digestibility of P linearly increased in both MSP (P = 0.038; 37.41, 48.14, and 42.35 %) and SLS (P = 0.002; 37.41, 46.07, and 43.776 %) diets, but not with TLA (P = 0.256; 37.41, 42.975, and 40.51 %). Data from this study suggest that bioavailability of P in TLA and SLS is lower compared to MSP, however, it can still be utilized effectively as an alternative source of P.

Bioavailability of Phosphorus in Turkey Litter Ash and Dried Swine Lagoon Sludge Fed to Growing Pigs

by
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BIOGRAPHY

Cooper McAuley was born October 17th, 1997, to Philip and Lynne McAuley in Concord NC. Being the first generation born off the farm, he still had a life around production agriculture through the experiences at his grandparents and uncle's farm's. This led him to start a venture in raising and showing sheep with the assistance of his grandfather, Thomas Lowder. Growing up, he learned more and more about how proper nutrition could lead to success in the show ring, and this is what sparked interest in animal nutrition.

Fast forward and he would go on to attend North Carolina State University for an undergraduate degree in poultry science. He would participate in multiple actives such as undergraduate research, summer internships, and collegiate livestock judging. Upon graduation Cooper would begin his Master of Science degree under the advisement of Dr. Eric van Heugten. He managed one study and would assist with several others to gain knowledge and experience in swine nutrition.

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I would like to take the time to show my appreciation to those who have helped me while perusing my master's degree. Through the long journey, you have all taken the time to help and encourage somehow along the way.

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Finally, I would like to say thank you to my family back home, especially my parents, Philip and Lynne, and my sister Ana, who always pushed me to accomplish my goals. I could not have completed this degree without the unconditional love and support you all always provided.

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CHAPTER 1 : Literature Review

Introduction

Promoting agricultural sustainability and limiting the carbon footprint of animal agriculture is one of the key goals in the industry today. Means of sustainability can vary, but in the swine industry, managing the excretion of nitrogen, phosphorus and microminerals such as zinc and copper is a priority. Formulating diets that reduce excess waste can also contribute to minimize other common issues like odor and ammonia emission. Although nutrients in waste can be limited by the use of efficient diets, there are still byproducts that are produced in animal production that have potential to be recycled. Using recycled minerals from animal and poultry waste provides the opportunity to accomplish the objective of sustainability while also providing ethical, economical, and effective feed inputs. This is not a new idea as recycling nutrients from animal waste has been practiced for decades through the use as fertilizer on crops. Midwestern states in the U.S have a higher level of nutrient balance through greater opportunities for land application of manure as a fertilizer. In North Carolina, there is a greater nutrient imbalance, as feed ingredients are imported, bringing nutrients into the state, resulting in excretion of nutrients in excess of what can be efficiently reused. This problem makes way for innovation of new methods of achieving sustainability, which include reusing these byproducts as a feed supplement. Phosphorus is the main nutrient of interest in this thesis, being the third most expensive nutrient considered when formulating swine diets (NRC, 2012). This literature review will provide background knowledge on phosphorus, its absorption and bioavailability, sources used in swine diets, and opportunities for recycled animal waste practices.

Phosphorus

Phosphorus was originally discovered in 1669 by Henning Brand (Wu, 2017), and it is known to be an essential macro mineral that has more functions than any other nutrient in the body. It is contained within every cell in the body with 80% stored in bones, while the remaining phosphorus serves in various metabolic pathways such as production of adenosine-triphosphate (ATP) and muscle metabolism (Takeda et al., 2012). Inorganic forms of phosphorus also found in cells can be used for acid-base balance, fatty acid transport, phospholipid formation and amino acid and protein synthesis (Jongbloed, 1987; NRC, 2012). Phosphorus itself is highly reactive and therefore is not found as a free element in the body. Therefore, in animals it is found as the anion phosphate (Crenshaw, 2001). Phosphate can have different forms, where the proton can move to different locations along the molecule. Phosphate is an ideal compound to attach to other elements such as calcium or iron. Phosphate regulation is maintained by the kidneys, bones and small intestine within the body (Cromwell, 2005).

Phosphorus is tightly correlated to calcium as phosphate typically binds to calcium to form calcium phosphate, found in bones (Crenshaw, 2001). This is one of the processes in biomineralization and sets the groundwork for the whole skeletal network. Biomineralization is the process of ions being stored to form solid minerals (Estroff, 2008). Inorganic phosphate can serve as transporter for iron and calcium ions throughout the body to perform a variety of functions.

Phosphorus absorption and digestibility

Phosphorus is digested throughout the body's entire intestinal tract, but the main site of digestion is the jejunum (Crenshaw, 2001). A study measuring digestibility of phosphorus

demonstrated a minimal role of the large intestine in absorption, showing no significant differences in ileal and fecal digestibility (Fan et al., 2001). Any undigested P that passes through the ileum is then excreted through feces (Liesegang et al., 2002). There are two main methods of phosphorus absorption which include passive transport and active transport within the small intestine (Breves and Schröder, 1991). Passive transport of P is dependent on electrochemical gradients in order to move inorganic P across epithelial cells lining the lumen of the small intestine utilizing paracellular movements (Sabbagh, 2011). Active transport utilizes sodium dependent transporters such as sodium-phosphate cotransporter type 2b, phosphate transporter-1 and phosphate transporter-2 (Adhikari, 2021). These transporters move P across the electrochemical gradients, depositing it to different locations within the body performing a metabolic function.

Homeostasis of P is regulated mainly by renal P excretion and intestinal absorption (Neer, 1979). Hormonal regulation of P metabolism is predominantly completed by three hormones including parathyroid hormone (PTH), calcitonin, and 1, 25 dihydroxycholecalciferol. PTH, produced by the thyroid gland, mainly affects P metabolism through its influence on kidney function (Lau et al., 1980). The rate of PTH secretion from the thyroid gland will be directly correlated to extracellular Ca concentrations while stimulating resorption of Ca and P from bone into circulation in times of low serum Ca (Jongbloed, 1987). Calcitonin does the opposite as it is stimulated by hypercalcemia, preventing resorption of Ca and P from bone (Jongbloed, 1987; Veum, 2010). Vitamin D (cholecalciferol) is an important nutrient to consider with absorption of P. Metabolically, provitamin D_3 (7-deydrocholesterol) is synthesized from cholesterol within the intestinal mucosa and this molecule then travels to the skin where it is converted to pre-vitamin D_3 with exposure to ultraviolet light (Jongbloed, 1987). Once absorbed

by the liver, vitamin D is hydroxylated creating 25-hydroxycholecalciferol which is then released into the circulation. The kidney is the site and rate limiting step where a 1α-hydroxyl is added to vitamin D, creating 1,25-dihydroxy vitamin D_3 which is its active form (Deluca, 2016). This is then released within the body to the main target tissues including the small intestine, bone, and kidney itself (Jongbloed, 1987). It can signal for increased intestinal absorption of P and in the case of low serum P, mobilize it from the skeletal network (Deluca, 2004). This also reduces the excretion of Ca and P from the kidneys (Petersen, 2004). Pigs must be given dietary vitamin D, because what is synthesized through ultraviolet light from sunlight is not sufficient to meet the vitamin D requirement of pigs, especially when housed in indoor commercial conditions.

Calcium (Ca) is the most abundant mineral throughout the body in terms of weight with 99% being found in bones and teeth (Vitti and Kebreab, 2010). Both Ca and P are involved in the formation of hydroxyapatite crystals which give bones structure and strength. Ca/P ratio in bones is 2:1 and serves as the main pool for these two minerals within the body. For the purposes of nutrition and bone mineralization, we must maintain proper ratios of Ca to P in the diet. Ca fed in excess can bind with endogenous P in the gastrointestinal tract leading to a decrease in reabsorption of endogenous P (Rutherford et al., 2004), meaning a high Ca/P ratio can decrease digestion of P. In the presence of phytate in the diet, calcium can also form insoluble Ca-phytate-P compounds that reduce effects of phytase on hydrolyzing P from phytate. (Dersjant-Li et al., 2015). Conversely, having too low of a ratio can decrease bone mineralization (Létourneau-Montminy et al., 2010). Lagos et al. (2019) showed standardized total tract digestible (STTD) Ca to STTD P value of 1.23:1 provided proper bone mineralization without being counterproductive to growth, which coincides with NRC (2012) recommendations. Vier et al. (2019) demonstrated the importance of considering the Ca/P ratio on a STTD basis. Researchers found that pigs

weighing 26 to 127 kg could maximize growth without sacrificing bone ash at a ratio between 1.75:1 and 1.82:1 STTD Ca:STTD P (Vier et al, 2019). Other minerals that can cause adverse effects on P absorption through forming insoluble compounds are Fe, Al, and Mg (McDowell, 1992).

A deficiency of P can lead to multiple issues in growing pigs. As stated before, P plays a vital role in bone development so a lack of P can lead to weaker bone strength (Veum, 2010). In growing pigs when they are still developing, a deficiency of vitamin D, Ca, and P can lead to rickets, which is the bending of legs (Cromwell, 2005). Other effects of P deficiency include reduced growth and feed utilization within the body.

Bioavailability and Digestibility

Digestibility of a mineral is the difference between dietary intake and fecal excretion (Jongbloed et al., 1991). There are several measurements to calculate aspects of digestibility and when put together can give strong evidence to show how much of a nutrient becomes absorbed within the body. Perhaps the simplest way is to calculate apparent total tract digestibility (ATTD). This is often presented as a percentage of the nutrient that is digested and is defined by total intake minus fecal excretion relative to total intake represented below.

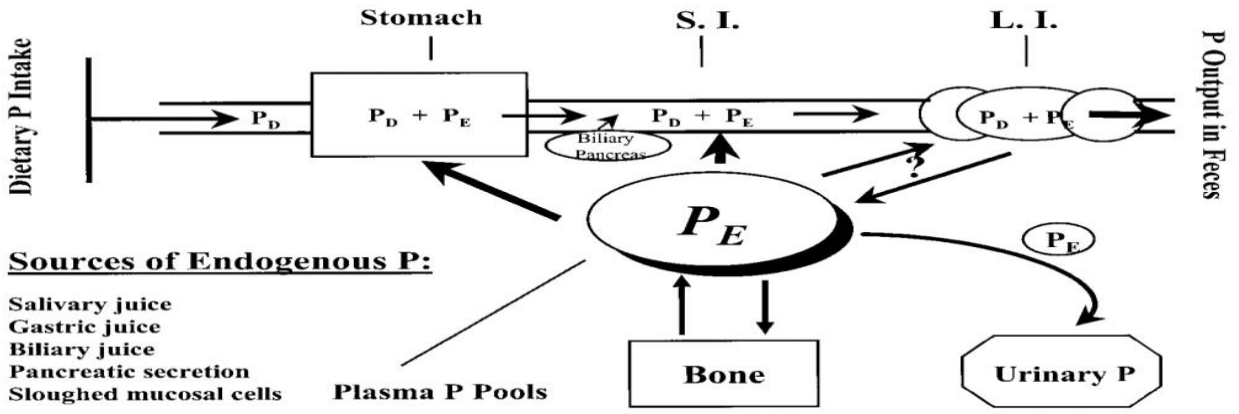
$$ATTD = \frac{intake - fecal\ excretion}{intake} * 100$$

However, this method alone is often criticized as it does not account for the endogenous losses of nutrients. The method that corrects for this is commonly labeled as true digestibility (Ammerman, 1995).

$$True\ Digestibility = \frac{intake - (fecal\ excretion - endogenous\ loss)}{intake} * 100$$

True digestibility provides the highest degree of accuracy amongst measurements; however, it is the least practical because it is difficult to measure. Figure 1 illustrates the flow of P within the body, both from dietary and endogenous sources. Endogenous losses include nutrients excreted from the body via feces or urine not from dietary origin. These endogenous losses mainly consist of gastric juice, saliva, intestinal cells, and pancreatic and bile secretions that enter the gastrointestinal tract (Vitti and Da Silva Filho, 2010).

Figure 1. Representation of whole-body P flow with emphasis on the gastrointestinal tract in pigs (Fan et al., 2001). Pd, Phosphorus of dietary origin; Pe, phosphorus of endogenous origin; S. I., small intestine; L. I., large intestine.



There are two categories of endogenous losses, being basal and specific losses (Stein et al. 2007). Basal endogenous losses are independent of feed ingredient type and remain constant. This means they can be more easily determined. Specific endogenous losses on the other hand are variable based on the characteristics and composition of ingredients used in the diet, causing total endogenous losses to be more difficult to calculate (Stein et al. 2007). Standardized digestibility measurements have been developed that consider basal endogenous losses, demonstrated in the equation below (She et al., 2017).

$$STTD = \frac{intake - (fecal\ excretion - basal\ endogenous\ loss)}{intake} * 100$$

By using an approximate value of 190 mg P per kg of dry matter intake as the basal endogenous P loss, the STTD values from ingredients can then be calculated when the ATTD values are known (NRC, 2012). STTD values are more additive and therefore lead to more accurate diet

Table 1: Digestibility values of common P sources (NRC, 2012; Kwon and Kim, 2017)

Item	DCP	MDCP	MCP	TCP	MSP
Total	18.8	-	21.5	17.7	24.7
ATTD	78.4	78.8	85.9	65.2	87.3
STTD	87.0	86.5	93.0	71.3	94.9

[1]DCP, dicalcium phosphate; MDCP mono-dicalcium phosphate; MCP, monocalcium phosphate; TCP, tricalcium phosphate; MSP, monosodium phosphate; ATTD apparent total tract digestibility; STTD, standardized total tract digestibility.

formulations compared to using ATTD values, preventing excess P excretion (She et al., 2017). Table 1 summarizes and compares the various digestibility values in common inorganic P ingredients used in diet formulation.

Bioavailability is the measurement of nutrient absorption and utilization throughout the body in physiological functions (Ammerman, 1995). Relative bioavailability provides estimates of the utilization of a given nutrient source when compared to a standard source. Specifically, regarding the bioavailability of P, monosodium phosphate (MSP) is often used as a standard given its high P digestibility (Table 1, NRC, 2012; Peterson et, al. 2011). Common forms of measurement to determine bioavailability is through that of the slope ratio assay (Littell et, al. 1997). To measure bioavailability of P using the slope ratio assay, multiple diets must be formulated with increasing concentrations of P, creating a standard line to which the source of P with unknown bioavailability can be compared. The ratio between the slope of the line of the test material and the slope of the standard line represents the estimate of bioavailability. The outcome variables to be measured in the evaluation of bioavailability vary depending on the nutrient under investigation. In the case of Ca and P, bone composition (total weight, bone ash, bone Ca, or bone P) and bone strength are commonly used (Ammerman, 1995). Lee et al. (2021) demonstrated that ash composition from the metacarpal and tibia is a better representation of bone ash throughout the body compared to the femur or rib. Bone strength is defined has the force needed to fracture a bone. In laying hens, it has been shown that bone breaking force has been linked to increased bone ash when fed varying levels of Ca and P (Wilson, 1991). If P from a P source is more available, it can deposit more P into the bone, therefore increasing its strength. When utilizing such a measurement in research studies, a longer amount of time is required for

differences to be detected compared to digestibility measurements. Growth performance parameters are also commonly used to estimate the absorption and bioavailability of P.

Phosphorus Requirements

The requirements of phosphorus in swine diets are variable at different points along the growth curve. Growing animals require a higher amount of phosphorus as their skeletal system is still developing. Sex can also play a role as gilts have a higher P requirement when compared to barrows (Ekpe et al., 2002), which may be related to increased lean tissue deposition in gilts vs. barrows, which increases P requirements. Table 2 lists the NRC (2012) phosphorus requirements of growing pigs at different weight intervals, using either standardized total tract digestibility (STTD), apparent total tract digestibility (ATTD), total P, and available P as a percentage. Rather than expressing P requirements on a total P basis, recent research supports inclusion rates based on STTD P as the most accurate standard (Cromwell, 2005). More recent studies completed by Vier et al. (2019) found that STTD P requirements listed in NRC (2012) are underestimated. It was determined that maximizing growth performance, carcass quality and bone mineralization in pigs weighing 24 to 130 kg required 116-131% of the NRC (2012) estimated value (Vier et al., 2019). When determining dietary requirements for P it is crucial to remember we must not only measure growth performance, as this value will be lower than that of maximizing bone mineralization (Crenshaw et al., 1981). Adeola et al. (2015) concurred with this notion and found STTD P requirement for supporting bone mineralization in femurs and metacarpals was 4.28 to 4.34 g/kg, whereas STTD P for ADG as criteria was 3.98 g/kg for pigs weighing 20 kg. Wu et al. (2019) also found that maximizing bone mineralization required a higher amount of STTD P compared to growth performance.

Table 2. Phosphorus Requirements of Growing Pigs When Allowed Feed Ad Libitum (90% dry matter)[1]

	Body Weight (kg)						
%	5-7	7-11	11-25	25-50	50-75	75-100	100-135
STTD Phosphorus	0.45	0.40	0.33	0.31	0.27	0.24	0.21
ATTD Phosphorus	0.41	0.36	0.29	0.26	0.23	0.21	0.18
Total Phosphorus	0.70	0.65	0.60	0.56	0.52	0.47	0.43
Available Phosphorus	0.55	0.40	0.32	0.23	0.19	0.15	0.15

[1]Adapted from NRC (1998 and 2012)

Data collected suggested that maximizing bone mineralization would require 140% of NRC (2012) recommendation in diets without supplemental phytase, and 170% in phytase containing diets (Wu et al., 2019). Partanen et al. (2010) reviewed phosphorus requirements due to recent advancements in genetics and found that feeding a rate of nonphytate P at 2.5 g/kg of BW resulted in both desired growth, bone mineralization, and bone strength. There are numerous other considerations that have potential to affect P requirements in pigs such as disease or environmental factors (Zhai et al., 2022). Saraiva et al. (2012) showed that pigs under heat stress conditions had a lower P requirement due to decreased in feed intake and growth.

Phosphorus Sources

Phosphorus is the first main macro-mineral considered when formulating swine diets. Phosphorus stored in plants is stored in the form of phytate, which is highly unavailable to monogastric animals. The six inorganic molecules of phosphorus attached as phosphate groups

on the myo-inositol ring of phytate can only be made available by an enzymatic reaction. Swine and other monogastric animals do not produce adequate amounts of phytase in their intestinal tract to liberate meaningful quantities of phosphorus. Therefore, in order to use phytate bound P in dietary ingredients, exogenous phytase would have to be supplemented. Supplementing dietary phytase in feed has been proven to increase both growth performance and feed efficiency throughout multiple stages of growth (Holloway et al.,2018; Braña et al., 2006). Apparent P digestibility has been shown to increase up to 24% through the addition of phytase in the diet (Simons et al., 1990). The use of microbial phytase has been shown to increase digestible P by up to 0.17% depending on the concentration in the diet, source of ingredients, inclusion of minerals, vitamin D concentration, age and species of the animal (Adeola and Cowieson, 2011). Super-dosing phytase is defined as utilizing 3 times the suggested amount (≥ 2500 FTU of phytase per kg of feed) as a means to further improve performance, largely independent of P release (Humer et al., 2015). More information on phytase and inclusion strategies can be found in a review article by Humer et al. (2015). Phytase supplementation alone does not eliminate the need for inclusion of P from inorganic sources, such as monocalcium phosphate (MCP), dicalcium phosphate (DCP) and monosodium phosphate (MSP). The composition of these inorganic ingredients can vary as they are not chemically pure compounds (NRC, 2012). Peterson et al. (2011) demonstrated relative bioavailability utilizing common inorganic sources of phosphorus. Using a slope ratio assay and monosodium phosphate as the standard, they found that pure monocalcium phosphate was 109% available based on metacarpal breaking strength and DCP was 57% available when compared with MSP (Peterson et al., 2011). They also found that different purity levels of MCP did not affect total P availability in growing pigs. Another study aimed to compare both apparent total tract digestibility and standardized total tract

digestibility of P in various P sources and showed that within both measurements, phosphorus in MSP (STTD: 94.9%, ATTD: 87.3%) was more available than that of MCP (STTD: 93.0%, ATTD: 85.9%) and DCP (STTD: 87.0%, ATTD: 78.4%) (Kwon and Kim, 2017). Understanding P requirements and the bioavailability of P sources can reduce both cost and potential excess P waste, leading to less environmental concerns. Cost of the various P sources is another important consideration. Listed below are prices of common inorganic P ingredients used when formulating swine diets. It compares prices per US ton, but also the cost on an individual total and STTD P basis in the current market.

Table 3. Cost of common phosphorus sources used in swine diets[a]

Phosphorus Source	Price Per Ton, $	Price per lb of P, $	Price per lb of STTD P, $
Monosodium Phosphate	3020	5.85	6.16
Monocalcium Phosphate, 21% P	1176	2.80	3.01
Dicalcium Phosphate, 18.5% P	1131	3.06	3.51
Defluorinated Phosphate, 18% P	1250	3.47	-[b]

[a] Prices on 6/28/2022
[b] STTD P$ value unavailable for defluorinated phosphate

Recycling Animal Waste

The animal agricultural industry is always striving to become more efficient and sustainable through various means. One of these methods includes the recycling of minerals contained in animal waste. Animal waste can contain high mineral concentrations that have potential to provide value as a recycled product. Fertilizer is the most common practice used to

put N and P back into the nutrient cycle. Fertilizer is utilized in crop production to supply nutrients required for growth. Animal waste has been used for decades as a source of N, P, K, Ca, Mg, and other micronutrients (Burns et al., 1985). Turkey litter is a prime example that has proven to be an effective fertilizer for crops as it contains several high concentrations of N and P (Zhang et al. 2013). Work conducted showed that composted turkey litter had a better P release than that of synthetic fertilizer and could be used as a replacement for conventional sources of P (Kraus and Warren 2000). Waste from hog farms held in anaerobic lagoons can also be used as a fertilizer on crops. The top layer of lagoons containing effluent are often drained and sprayed as a nutrient source for plants and soil providing key nutrients. Adeli and Varco (2001) found no significant difference in dry matter yield of summer forage grasses for those sprayed with swine effluent or a commercial fertilizer. This shows how effective recycling swine waste can be as a nutrient source for crops, when applied at appropriate levels. All swine producers will have a manure management plan that is commonly required implemented at their facilities. This involves knowing the nutrient content of the manure and soil concentrations of potential sites of application. Timing of application can also play a role; manure application in the fall allows more time for the organic contents of manure to break down before crop utilization compared to spring application (Hernandez and Schmitt, 2012). In many states N and sometimes P levels are monitored for land application due to potential environmental concerns. If soil P levels are higher than what crops can absorb, this potential environmental hazard leads to excess P runoff in surface waters (Delaune et. al. 2004). The accumulation of waste from animal production provides other opportunities, such as an energy source when combusted for heat. Burning manure products such as poultry litter provides incentives in the form of energy credits, which provide financial benefit to producers. This process produces an inorganic, sterile ash by-

product. This ash is a concentrated source of minerals and is a potential plant fertilizer. A study conducted using corn as the crop of interest showed that ash can be utilized as a non-organic source of P, while also providing trace metals at levels that are within acceptable ranges (Pagliari et al.,2010).

Ash as a Feed Ingredient

Research using ash as an animal feed additive providing primarily P has been limited but some work has been completed to determine if there is any potential value of ash as a P source. In commercial broilers, poultry litter ash was compared to dicalcium phosphate to investigate effects on growth performance and carcass traits (Blake and Hess, 2014). The authors reported that there were no negative effects on growth or feed conversion in birds when P from dicalcium phosphate was fully substituted with P from ash. Akpe (1984) determined the effect of four different poultry ash residues produced from a fluid bed reactor as a P source compared to calcium phosphate monobasic monohydrate in male turkeys. A slope ratio assay was employed using bone ash as the variable, while linearly increasing P inclusion from the assigned source. Bone ash increased with higher P inclusion, but the four ash samples had significantly lower levels of bone ash compared to calcium phosphate monobasic monohydrate. The four ash sources had bioavailability values of 80.6, 87.5, 77.6, and 72.4% compared to calcium phosphate monobasic monohydrate, which was set at 100% (Akpe, 1984). Previous research conducted at North Carolina State University evaluated an ash source produced from gasification of turkey litter, which resulted in an analyzed P concentration of 6.09%. It was then evaluated as a P source when fed to growing pigs compared to a diet deficient in P and a positive control diet with

P supplemented from dicalcium phosphate. ATTD of P from pigs supplemented with turkey litter ash was 55.7% compared to those fed dicalcium phosphate at 48.5% (van Heugten et al., 2008).

Dried Swine Lagoon Sludge

It is common practice in North Carolina to collect hog waste in lagoons. These anaerobic ponds collect both liquid and solid forms of waste which can later be used as nutrient source primarily for crops or pastures. Anaerobic treatment of swine manure is dependent on facultative anaerobic and anaerobic bacteria to promote breakdown into gas, cellular biomass and sludge material (Owusu-Twum and Sharara, 2020). Over time, there will be different layers within the lagoon as displayed in Figure 2. Earlier it was mentioned that the top layers of lagoons are commonly used as fertilizer sources to take advantage of their mineral concentrations. At the bottom layer, there is accumulation of a concentrated, more solid layer termed sludge. This mineral rich material accumulates over time and must be cleaned out periodically for farmers to stay in compliance with permits and maintain lagoon performance. Once collected, the sludge can then be dried into a final granular form. Lagoon sludge composition can vary between sources. Owusu-Twum and Sharara (2020) compiled data from 26 sampled lagoons and the nutrient profiles of the collected sludge (Table 4). Land application of animal manure is normally regulated on a N basis, however with the high concentration of P in sludge, it then becomes the limiting nutrient for application (Schmidt and Engineer, 2013).

Figure 2. Single stage anaerobic manure treatment lagoon. From Owusu-Twum and Sharara (2020).

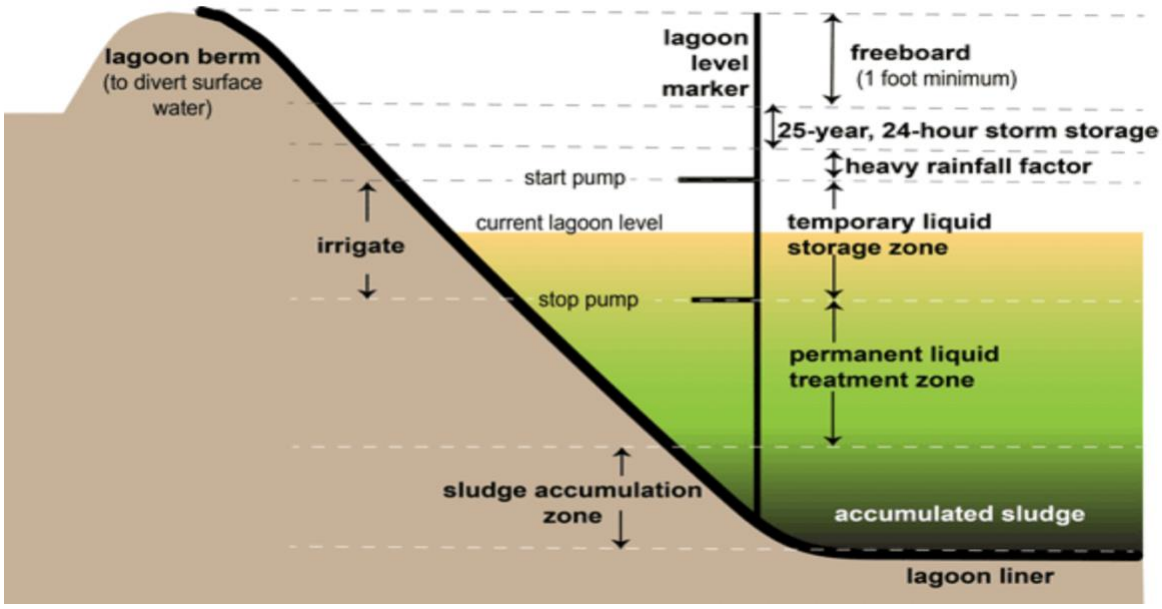


Table 4. Composition of swine lagoon sludge

Parameter	Mean	StDev	Range	# of lagoons
pH	7.37	0.42	7.0-7.6	26
P (g/l)	3.83	4.58	0.6-7.3	27
Ca (g/l)	4.49	5.12	1.2-7.5	17
Mg (g/l)	1.22	1.46	0.2-2.8	26
K (g/l)	0.62	0.32	0.4-1.1	26
NH_3-N (g/l)	0.86	0.87	0.5-1.5	15

From: Owusu-Twum and Sharara (2020).

Another process used to recycle swine manure solids is through drying and combusting as a source of energy through heat. The ashed byproduct has a yield of approximately 59 kg

produced for every 454 kg of combusted dried swine lagoon sludge. Ashing this product destroys any bacteria or viruses from the sludge thus creating a sterile and concentrated mineral source. van Kempen (2002) showed the composition of ashed swine manure solids collected from a belt system to be 11.5% Ca, 13.3% P, 12% K, 0.18% Zn, 0.05% Cu, and 0.73% Fe. Ash from swine lagoon sludge can serve as a fertilizer, but no research has been conducted to evaluate it as a potential mineral source for swine. However, van Kempen (2002) evaluated ash derived from the combustion of swine manure solids collected from a belt system to determine its value as a mineral supplement. Diets supplemented with this ash source were compared to a standard diet that was supplemented with minerals from limestone, DCP, potassium chloride, and salt. P from ash supplemented diets had an ATTD value of 63% compared to the control, which had an ATTD value of 72.3% (van Kempen, 2002). The P concentration in the ash used in this study was higher than expected, and therefore the experimental diet contained 40% more P than desired, which could potentially have resulted in an underestimation of P digestibility (van Kempen, 2002). Similarly, work completed by van Heugten et al. (2008) compared the ATTD between different sources of ash. These sources included ash from incinerated swine mortalities from a grower-finisher facility, and gasification of swine manure solids from two separate facilities. Results showed that ATTD of P ranged from 40.4 to 51.6% between different ash sources compared to the DCP diet which had a digestibility of 48.5%. Characteristic of ash products can vary depending on raw material sources and processing methods, which can subsequently impact P digestibility. van Heugten et al. (2008) reported a second study to compare ash from gasification of swine waste solids to a negative control diet (no supplemental P) and a positive control diet containing P from DCP. When fed for a short duration, ash had no effects on ADG or ADFI, but gain/feed overall tended (367 vs. 351 g/kg) to be better for pigs

supplemented with DCP. Serum P levels were greater for pigs fed DCP (8.59 mg/dL) compared to ash (7.93 mg/dL), but both groups supplemented with P had higher concentrations of serum P than the negative control (7.22 mg/dL). However, bone ash was not different between treatments (van Heugten et al., 2008). The authors concluded that overall P digestibility from ash is heavily dependent of the source but still shows promise as an alternative source of P.

Conclusion

Phosphorus is the most expensive macro-mineral when formulating swine diets and plays a crucial role physiologically. However, when excess P is excreted through feces and urine it can potentially give rise to environmental concerns. New efforts are being made to try and utilize recycled byproducts from animal agriculture and find alternative solutions with the goal of being more sustainable. Recycling minerals derived from animal and poultry wastes back into the animal food chain provides realistic economic and environmental opportunities. The goal of the current research project was to examine the value of two recycled materials as a P source for swine.

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Chapter II:

Bioavailability of Phosphorus in Turkey Litter Ash and Dried Swine Lagoon Sludge Fed to Growing Pigs

Introduction

Phosphorus (P) is an expensive feed ingredient in swine diets that is not only crucial for bone mineralization, but also numerous other metabolic functions (Cromwell, 2005). When fed in excess, it leads to excess P in manure, potentially leading to environmental hazards. Even with balanced diets, animal waste byproducts contain significant amounts of minerals. Within the agricultural industry there is a push to become more sustainable, reusing any nutrients possible through different stages of production. These mineral rich products derived from animal manure are often recycled through the use as fertilizer, primarily for its nitrogen and P concentrations. However, the concentrations of nutrients utilized for land application is strictly regulated to avoid potential runoff contaminating water sources (Delaune et al., 2004). Therefore, it is vital to find new innovations in which to utilize this nutrient source. One avenue is through combusting of this waste as a heat source, leaving an inorganic, sterile ash product that has the potential of being recycled back into animal feed. Limited research has been conducted in utilizing ashed animal waste byproducts as a mineral source in swine diets. Two different products were tested in the current study: turkey litter ash (TLA) and dried swine lagoon sludge (SLS).

TLA is the inorganic byproduct of burning turkey litter as a heat energy source. Studies have shown that turkey litter ash as a P source did not negatively affect the growth performance of broilers compared to those fed dicalcium phosphate (Blake and Hess, 2014). van Heugten et al. (2009) demonstrated apparent total tract digestibility (ATTD) of P to be higher in pigs supplemented with turkey litter ash (55.7%) compared to pigs fed dicalcium phosphate (DCP) (48.5%). SLS is collected from the bottom of lagoons, and unlike traditional fertilizers, it is limited for its land application not by nitrogen content, but by its high P concentration (Owusu-Twum and Sharara, 2020). In previous research, when ash from swine manure solids was utilized

as an alternative P source, ATTD of P was lower (63%) than that in pigs supplemented with DCP (72.3%) (van Kempen, 2002). Source of the waste byproduct and combustion procedures used have also shown to affect mineral concentrations and their digestibility when fed to growing pigs (van Heugten et al., 2009).

The goal of the current project was to evaluate these two alternative recycled P sources on growth performance and to determine the bioavailability of P using a slope ratio assay. TLA and SLS were compared to monosodium phosphate (MSP) as a standard with an assumed bioavailability of P of 100% (NRC, 2012; Peterson et, al. 2011).

Materials and Methods

The research protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee at North Carolina State University, Raleigh, North Carolina.

Animals, Housing and Experimental Design

Fifty-six crossbred pigs (Smithfield Premium Genetics) with a mean BW of 34.92 ± 0.29 kg were used. All pigs were housed at the North Carolina State University Swine Nutrition Barn (Raleigh, NC) in 2 rooms with 28 pigs in each room. The rooms were environmentally controlled, with an end-wall baffle ventilation system and a pit storage system for waste storage and removal. Pigs were housed individually in pens (1.8 x 0.94 m) and blocked based on initial BW (8 blocks per treatment). Pens were then randomly allocated within blocks to one of 7 experimental treatments. Pigs were limit-fed daily at 3 times maintenance calculated as 197 kcal ME/kg $BW^{0.60}$ (NRC, 2012), based on the mean BW of each block. Pigs were fed twice daily at 8:00 and 16:00 and had unlimited access to water.

Diet formulation and Sample Collection

Samples of turkey litter ash (TLA) and dried swine lagoon sludge (SLS) were submitted to the New Jersey Feed Laboratory, Inc (Trenton, NJ) for the analysis of concentrations of phosphorus (P), calcium (Ca) and sodium (Na). Analyzed concentrations (as-is basis) of Ca, P, and Na were 8.31, 3.98, and 0.70, respectively for TLA and 5.81, 4.31, and 0.12 for SLS and these values were used in the formulation of the experimental diets.

Dietary treatments consisted of a negative control diet, deficient in P (40% of the suggested requirement from NRC 2012) and negative control diets with supplemental P at 0.075%, or 0.150% from either monosodium phosphate (MSP), TLA or SLS (Table 1). MSP was used as a standard with a reported P availability value of 100% (NRC, 2012; Peterson et, al., 2011). Experimental diets were created from a corn-soybean meal basal mix that met all nutritional requirements per NRC (2012) except for P, Ca, and Na. The basal mix was divided into 7 portions to which calcium carbonate, P source, salt, or fine builder sand were added to manufacture the final experimental diets. Calcium carbonate was used at appropriate levels to maintain a constant Ca:P ratio of 1.4:1, which was the lowest ratio that could be achieved in the formulation. Salt was added to maintain consistent Na levels, to account for the Na derived from MSP. Builder sand was used as a filler to maintain equal inclusion of ingredients across diets. Titanium dioxide was included in all diets at the rate of 0.3% as an indigestible marker to determine total tract nutrient digestibility. All diets were manufactured at the North Carolina State University Educational Feed Mill Education Unit (Raleigh, NC) and presented as a meal form. Samples of all seven diets were taken and submitted to the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratory for chemical verification.

Pigs were weighed individually on day 0, 14, and 28. Pigs were observed daily to ensure they were healthy, active, consumed all their allotted feed and had free access to water. During the last three days of the study, fresh fecal samples were collected each day and pooled within each pig and stored at -20 ℃ for further processing. Pooled samples were then dried at 64℃ for a minimum of 72 hours in a conventional drying oven and weighed daily until weights no longer decreased, indicating that all moisture had been removed. Samples were then sealed in plastic zip-lock bags and stored in a climate-controlled room until they were ground to a uniform size. On the final day of the trial (day 28), pigs were euthanized via captive bolt gun followed by exsanguination. Blood samples were taken at this time in tubes (Vacutainer, Becton, Dickerson and Company, Franklin Lakes NJ) for the separation of serum by centrifugation at 900 x g for 20 min at 25˚C. Serum samples were then stored in -20˚C until further analysis. Organs (heart, liver, spleen and kidney) were excised, collected and weighed for each pig. The right front foot of each pig was collected and stored at -80˚C until the 3rd metacarpal bones were dissected for the determination of bone composition and strength.

The 3rd metacarpal bones were isolated and then cleaned off with a scalpel. Bone length was measured with a digital caliper before being evaluated for bone strength. Bone strength was determined with texture analyzer equipment (Model: TA.XT Plus, Texture Analyser Technologies, Scarsdale, NY, USA), using a three-point bending test with 5 cm between the supports and a 250 kg load cell. Peak breaking strength was defined as the maximum load supported before failure. After breaking, bones were defatted by soaking in petroleum ether for 24 hours. They were then transferred into a crucible and weighed before placing them in a muffle furnace where they were ashed at 600˚C for 10 hours. Ash weights were then recorded and calculated as the percentage of deffated bone weight. Two grams of ash were then digested by

boiling in 20 mL of 4 N HCL. Digesta was then filtered into 100 mL of deionized water and samples were stored for subsequent mineral analysis**.**

Chemical analyses

Samples of turkey litter ash (TLA) and dried swine lagoon sludge (SLS) were submitted to the New Jersey Feed Laboratory, Inc (Trenton, NJ) for the analysis of concentrations of phosphorus (P), calcium (Ca) and sodium (Na). Analyzed concentrations (as-is basis) of Ca, P, and Na were 8.31, 3.98, and 0.70, respectively for TLA and 5.81, 4.31, and 0.12 for SLS. Feed samples and fecal samples were submitted to the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO) for proximate analyses, (AOAC Official Method 990.03, 2006), P (AOAC Official Method 966.01, 2006) Ca, Mg, Fe, Cu, and Zn (AOAC Official Method 980.02, 2006) and titanium dioxide (Myers et al., 2004). Apparent total tract digestibility was calculated for P, Ca, Mg, Fe, Cu and Zn using the index method (Adeola, 2001) where titanium dioxide served as the indigestible marker. One set of serum samples were analyzed by Antech Diagnostics (Cary, NC) for serum chemistry (total protein, albumin, globulin, albumin/globulin ratio, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatatse, gamma-glutamyl transferase, blood urea nitrogen, creatine, phosphorus, glucose, calcium, magnesium, sodium, potassium, sodium/potassium ratio, chloride, cholesterol, triglyceride, amylase, precision pancreas-specific lipase, creatine phosphokinase) and a complete blood count (white blood cells, red blood cells, hemoglobin, hemocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet, neutrophils, lymphocytes, monocytes eosinophils, and basephils). Duplicate serum samples were submitted to the Environmental and Agricultural Testing Service laboratory (EATS) at the

Department of Crop and Soil Sciences at North Carolina State University. These samples were analyzed for P, Ca, Mg, Fe, Cu, and Zn using Ion Coupled Plasma Spectrometry (ICP-OES). 15 mL sub-samples of bone ash digestate were analyzed for Ca and P by the Environmental and Agricultural Testing Service laboratory (EATS), Department of Crop and Soil Sciences, at North Carolina State University using ICP-OES.

Calculations and Statistical Analysis

Statistical Analysis was conducted using the PROC GLM feature of SAS (SAS Inst. Inc., Cary., NC) as a randomized complete block design. Individual pig was the experimental unit for performance, serum chemistry, bone composition, bone strength and digestibility measurements. The model included effects of block and P supplementation. Multiple linear regression and slope ratio assays were used to estimate bioavailability of P compared to the standard. Statistical significance was declared at $\alpha \leq 0.05$ and tendencies considered $0.05 < \alpha \leq 0.05$.

Results

Growth Performance

Average daily gain during the first 2 weeks of the study was not different between pigs supplemented with P from either MSP (P = 0.176), TLA (P = 0.256) or SLS (P = 0.887) (Table 2). However, during the second 2-week period, ADG linearly increased with supplemental P from MSP (P = 0.004) and tended to increase when P was supplemented from SLS (P = 0.069). Overall, ADG linearly increased when pigs were supplemented with P from MSP (P = 0.022), tended to increase with P from TLA (P = 0.072), but not with P from SLS (Figure 1). Final body

weight tended to increase with P supplementation from MSP (P = 0.085) but was not affected when P was supplemented from TLA or SLS.

For organ weights expressed as an as-is weight, kidney weight tended to decrease in pigs supplemented with MSP, but the weight of the liver, spleen, and the heart were not impacted (Table 3). Kidney weight relative to BW linearly decreased (P = 0.041) with increasing MSP and heart weight relative to BW tended to decrease with increasing TLA (P = 0.072). Other relative organ weights were not impacted.

Serum Chemistry

Analysis of serum P using a serum chemistry panel (Table 4) showed that serum P linearly increased (P = 0.007) with increasing P from MSP (8.76, 9.08, and 10.00 mg/dL), but not with supplemental P from TLA (P = 0.232; 8.76, 8.55, and 9.28 mg/dL) or SLS (P = 0.247; 8.76, 9.18, and 9.25 mg/dL) (Figure 2). Serum Ca concentrations linearly decreased (P<0.030) with increasing P from MSP, TLA, and SLS. Albumin to globulin ratio tended to linearly increase with supplemental P from TLA (P = 0.053) and SLS (P = 0.060). Serum creatine (P = 0.0279) and triglyceride (P = 0.017) concentrations linearly increased with increasing P from MSP. Pigs fed SLS diets tended to have decreased levels of serum potassium (P = 0.052) and this led to a significant decrease in the sodium to potassium ratio (P = 0.023). SLS fed pigs also had a linear increase in serum cholesterol concentrations (P = 0.017).

*S*erum mineral concentrations were also determined using traditional wet chemistry methods (Table 5) and showed that serum P increased linearly in pigs supplemented with P from MSP (P = 0.006; 16.11, 16.53, and 18.37 mg/dL), and tended to increase in pigs fed P from SLS (P = 0.094; 16.11, 16.76, and 17.11 mg/dL); however, serum P was not significantly different

with P supplementation from TLA (Figure 3). Serum Ca concentration decreased in pigs fed supplemental P from MSP (P = 0.023) and tended to decrease when P was supplemented from TLA (P = 0.060) and SLS (P = 0.060). Pigs fed SLS diets had a linear decrease in serum Mg concentration (P = 0.021; 2.49, 2.22, and 2.13 mg/dL) and increased serum Zn concentration (P= 0.014; 0.099, 0.135, and 0.113Mg/dL), but there was no difference in pigs fed MSP or TLA diets. Serum concentrations of Cu and Fe were not impacted by dietary treatments.

Bone Composition/ and Strength

Total ash weight of the third metacarpal bone linearly increased in pigs supplemented with additional P from MSP (P = 0.022; 3.62, 3.85, 4.04 g), TLA (P = 0.005; 3.62, 3.86, 4.05g), and SLS (P = 0.015; 3.62, 3.93, 4.05g) (Table 6). Ash weight as a percentage tended to increase in pigs fed supplemental P from MSP (P = 0.082; 40.28, 40.98, 43.62%) (Figure 5). Total bone P significantly increased in pigs fed added P from MSP (P = 0.017; 0.163, 0.169, 0.184g) and SLS (P = 0.028; 0.163, 0.167, 0.192g), and tended to increase with added P from TLA (P = 0.057; 0.163, 0.180, 0.180g) (Figure 6). Total bone Ca also increased in pigs supplemented with additional P from SLS (P = 0.052; 0.334, 0.344, 0.368) and tended to increase in pigs supplemented with MSP (P = 0.048; 0.334, 0.341, 0.387). There was no difference in bone P or bone Ca between treatments when expressed as a percentage. However, the Ca to P ratio in bone decreased significantly with increasing P from all sources used ($P \leq 0.039$). Peak bone breaking force linearly increased with the addition of P of MSP (P = 0.008; 65.53, 72.01, and 84.10 kg) and TLA (P = 0.015; 65.53, 64.95, and 81.06 kg), and it tended to increase with P from SLS (P = 0.077; 65.53, 71.49, 77.18) (Table 6). There was no difference in bone length regardless of treatment.

Apparent Total Tract Digestibility

Apparent total tract P digestibility increased linearly in pigs supplemented with P from MSP (P = 0.038; 37.41, 48.14, and 42.35 %) and SLS (P = 0.002; 37.41, 46.07, and 43.776 %), but was not affected by TLA (P = 0.256; 37.41, 42.975, and 40.51 %) (Table 7; Figure 7). Manganese digestibility linearly decreased with supplemental P from MSP (P < 0.001; 7.56, -1.91, and -8.18 %), TLA (P = 0.003; 7.56, 1.85, and -5.90), and SLS (P = 0.009; 7.56, 5.36, and 0.50). Cu digestibility linearly increased with MSP (P < 0.001; -0.38, -3.77, and 18.17), TLA (P = 0.004; -0.38, 9.83, and 11.52) and tended to increase with SLS (P = 0.088; -0.375, 8.66, and 2.80). Zn digestibility increased with supplemented SLS (P = 0.002; -27.12, -5.20, and -61.32) and tended to increase from TLA (P = 0.055; -27.14, -23.33, and -17.23), while it was not affected by MSP. Digestibility of Ca and Fe were not affected by increasing levels of MSP, TLA or SLS.

Bioavailability of P

Results comparing the ratio of the slopes of the test materials and the control providing relative bioavailability estimates are shown in Table 8. The relative bioavailability was calculated by taking the ratio of either the linear regression coefficient from TLA or SLS compared to the standard MSP slope. The slope ratio estimated values for ADG were 58.64% for TLA and 65.05% for SLS compared to MSP. Serum P performed by a serum chemistry panel showed the slope ratio for TLA was an estimated 41.39% and that for SLS was 52.47% compared to MSP. However, serum P concentrations performed using wet chemistry methods had estimated values of 37.28% for TLA and 54.09% compared to MSP. The slope ratio for bone ash (g) was 103% for TLA and 109%, however when taken as a percentage of total bone weight,

TLA was an estimated at 73.71% and 43.94% for SLS compared to MSP. Using the slope of each line, we calculated relative bioavailability of TLA to be 73.75% and SLS as 67.1% for peak breaking strength.

Disscussion

The objective of this study was to evaluate two recycled animal waste products, TLA and SLS, for their ability to provide a source of bioavailable P to growing pigs. Several different measurements were conducted, such as growth performance, serum chemistry, bone mineralization and ATTD of P, to provide a comprehensive evaluation of these waste materials as potential alternative P sources to be used in swine diets.

Growth performance and organ eights

In the current study, supplementing TLA and SLS as an alternative source of P did not impact growth performance of growing pigs. This concurs with research done by Blake and Hess (2014) who showed that replacing dicalcium phosphate (DCP) with combusted TLA did not compromise the growth of broilers. Inclusion of dietary P below the requirement will typically impair weight gain in swine over time (Cromwell et. al., 1972). Results from the present study concur with this notion as pigs supplemented with P from MSP, which is a highly bioavailable P source (NRC, 2012), tended to have a higher BW and ADG than those fed the negative control diet deficient in P. However, pigs supplemented with P from TLA and SLS had similar ADG than pigs for the negative control diet, suggesting the P from these sources was less bioavailable for growth compared to MSP. The only significant difference detected in organ weights was that of kidney weight in relation to BW in pigs supplemented MSP was greater than control pigs.

This is potentially due to the kidney playing an active role in the regulation of Ca and P metabolism (Jongbloed, 1987). Perhaps if the experimental window was greater than 28 days, a greater impact of P supplementation from the difference sources could have been detected for growth performance and organ weights. Another study feeding varying concentrations of P failed to detect any difference in growth in a time period similar to the one in the current study (Peterson et. al 2011).

Serum chemistry

Serum P concentrations increased with the inclusion of P, but this appeared to only be the case for MSP with a tendency for SLS. This suggests that the bioavailability of P from TLA and SLS are inferior compared to MSP. Serum P values for MSP fed pigs coincide with work completed by Coalson et al. (1972), demonstrating that increasing dietary P directly affects serum P levels. Serum P values were in a similar range with those in the study mentioned with differing dietary P inclusion levels. Serum Ca linearly decreased with the increasing inclusion of P from MSP, TLA and SLS. This was an unexpected finding as the Ca/P ratio was maintained with Ca from calcium carbonate, a highly available Ca source, as dietary P increased. Pigs fed P from MSP had linear increases in serum creatine, which is considered a sign of kidney malfunction (Whalan, 2015). This may be related, in part, to the decrease in kidney size in relation to BW in that treatment group. Pigs supplemented with SLS had linear increases in serum concentration of cholesterol while those fed MSP had an increase in serum triglyceride concentration. A study performed in broilers also found that increased dietary P increased these parameters (Li et al., 2016). Mineral analysis of other minerals besides Ca and P showed little difference due to P source and inclusion level. However, there was a liner decrease in serum Mg

and a tendency of decrease serum K. Mg and K concentrations were not corrected for in the experimental diets, so this potentially explains this decrease in serum concentrations.

Bone composition

Peak breaking strength is defined as the force necessary to break a bone when held in a horizontal position. The peak bone breaking strength values from this study were similar to those reported by Hall et al. (1991). It has been shown that increasing P in the diet linearly increased bone breaking strength (Adeola et al., 2015). In agreement, the current study demonstrated a linearly increase in peak breaking strength of the third metacarpal bones in pigs supplemented with P from MSP and TLA, with a tendency to increase peak breaking strength with P from SLS. These results suggest that all three sources of P were bioavailable. Bone mineralization is another good measurement for P bioavailability. Once metabolic P requirements are met within the body, the rest will be stored in bone. There was an increase in total bone ash by all P sources, with bone ash concentration only tending to increase from those pigs supplemented with P from MSP. There was a significant linear increase in total bone P in pigs supplemented with P from MSP and SLS and a tendency with TLA, but differences in bone concentrations of P and Ca were not detected. This is contrary to what has been reported in other studies where bone P concentrations increased with higher dietary P inclusions, but total weight of bone P was unaffected (Adeola et al., 2015; Peterson et al. 2011).

Apparent total tract digestibility

Digestibility of P linearly increased with the inclusions of P from MSP and SLS. Digestibility values for SLS and TLA were comparable to those reported in previous research by

van Heugten et al. (2009) feeding recycled animal waste products. However, they were lower than the reported digestibility for ashed swine manure solids reported by van Kempen (2002). Authors from those studies stated that the source and manufacturing technology used to produce these animal waste byproducts can potentially impact digestibility of P when fed to pigs. As P inclusion in diets increased, so did the amount digested in the GI tract for those fed MSP and SLS diets, which is in agreement with previous work (Patanen et al, 2010). The value for ATTD of P from MSP was lower than that reported by NRC (2012), although this value represents the digestibility of P in the entire diet. Nonetheless, this study it is still effective as a comparison for the relative bioavailability of the P sources under investigation. An interesting result showed that ATTD of P from SLS was very comparable to that of pigs supplemented with MSP. Digestibility of Mn linearly decreased with increasing P inclusion. This is potentially due to increased phosphates forming insoluble bonds with manganese. Digestibility of Zn significantly increased in pigs supplemented with SLS and tended to increase with TLA. This may be explained by the added concentration of Zn supplied from TLA and SLS.

Slope Ratio and relative bioavailability

The results from the slope ratio assay yielded bioavailability estimates ranging between 58.64 and 73.71% for TLA and 54.09 and 103.31 for SLS, depending on which variable was used to estimate bioavailability. Bone criteria are more responsive when evaluating dietary P (Koch and Mahan, 1985). The estimated bioavailability by use of a slope ratio assay showed P bioavailaility for TLA as 73.75% and for SLS at 67.1% using bone strength as measurement. For comparison, DCP has been reported to have a bioavailability of approximately 57% compared with MSP, using bone breaking strength as criteria (Peterson et al. 2011). This suggests that the two alternative P

sources evaluated in the current study may be more bioavailable to pigs compared with DCP, although this was not directly evaluated herein.

Conclusions

Compared to MSP, TLA and SLS was more limited as an alternative P source. However, it can still be used effectively, as it has shown to be comparable with DCP as an inorganic P source. This study was designed to evaluate bioavailability of P in these two recycled products. Relative bioavailability varied between criteria, however using peak breaking force in bone showed TLA as 73.75% and SLS at 67.1% available. However, ATTD of P from the diet with SLS closely resembled the value from the diet with MSP. More research needs to be conducted on SLS as a feed ingredient as it is still an organic material as used in this study. Though sources can vary, SLS has a more ideal ratio of Ca/P for inclusion into rations as well as providing other sources of minerals. The present study was not designed to specifically evaluate other microminerals, however we did observe increased ATTD values of Zn and Cu in those diets supplemented with SLS. If dried SLS was ashed, not only would it provide energy from heat, but the ashed end product would contain greater concentrations of minerals. Combusting SLS would also neutralize any organic material, resulting in a sterile and true inorganic source of P.

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Table 1. Composition and proximate analysis of experimental diets (as-fed basis)

Ingredients (%)	**Negative**	**MSP+0.075**	**MSP+0.15**	**TLA+0.075**	**TLA+0.15**	**SLS+0.075**	**SLS+0.15**
Corn (7.5% CP)	60.16	60.16	60.16	60.16	60.16	60.16	60.16
Soybean meal (47% CP)	30.65	30.65	30.65	30.65	30.65	30.65	30.65
Poultry fat	3.0	3.0	3.0	3.0	3.0	3.0	3.0
L-lysine HCL	0.22	0.22	0.22	0.22	0.22	0.22	0.22
L-Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Threonine	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Calcium carbonate	1.243	1.515	1.788	1.111	0.990	1.252	1.26
Salt	0.49	0.345	0.2	0.460	0.427	0.488	0.480
Trace mineral premix[1]	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Vitamin premix[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Titanium dioxide	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Sand	3.457	3.026	2.594	1.739	0.013	1.710	0
Monosodium phosphate	0.19	0.494	0.798	0.19	0.19	0.19	0.19
TLA	0	0	0	1.880	3.760	0	0
SLS	0	0	0	0	0	1.740	3.480

Table 1 (continued)

Ingredients (%)	Negative	MSP+0.075	MSP+0.15	TLA+0.075	TLA+0.15	SLS+0.075	SLS+0.15
Analyzed Composition							
Crude Protein, %	18.09	19.11	19.02	19.09	18.42	20.35	19.57
Moisture, %	11.64	11.44	11.47	11.62	11.72	11.78	12.01
Fat, %	4.39	4.46	4.21	4.14	4.15	4.16	4.50
Fiber, %	1.87	1.96	1.77	2.06	2.40	1.91	2.25
Ash, %	8.72	9.35	9.59	8.53	8.37	7.92	6.61
Calcium, %	0.738	0.8725	1.12	0.90	1.03	0.82	1.05
Phosphorus, %	0.359	0.49	0.53	0.44	0.51	0.45	.51
Manganese, ppm	48	44	43	77	108	64	87
Iron, ppm	245	217	210	277	382	434	651
Copper, ppm	12	12	16	35	59	30	47
Zinc, ppm	66	69	71	91	120	138	217
Titanium, ppm	1550	1570	1710	1625	1630	1560	1670

[1] Supplied per kg of complete diet: 33 ppm of manganese, 110 ppm of zinc, 110 ppm of iron, 17 ppm of copper, 0.30 ppm of iodine, and 0.30 ppm of selenium.
[2] Supplied per kg of complete diet: 6,614 IU of vitamin A, 1,323 IU of vitamin D3, 27 IU of vitamin E and 2.7 mg of vitamin K, 35 µg vitamin B12, 6.2 mg riboflavin, 20 mg pantothenic acid, 35 mg niacin, and 0.09 mg biotin.

Table 2. Response of growth performance to supplemental P source

		MSP				TLA				SLS			
	NC	+.075	+.15	SEM	P -value	+.075	+.15	SEM	P-value	+.075	+.15	SEM	P -value
BW, kg													
d 0	35.5	34.00	35.00	0.671	0.673	34.94	35.31	0.671	0.832	34.63	35.134	0.671	0.743
d 14	44.31	43.44	44.69	0.643	0.682	44.00	44.63	0.643	0.738	44.38	44.06	0.643	0.809
d 28	56.81	56.80	58.50	0.786	0.085	56.94	58.00	0.786	0.271	56.94	57.732	0.786	0.367
ADG, kg/d													
d 0 to 14	0.630	0.674	0.692	0.033	0.176	0.647	0.665	0.033	0.256	0.696	0.625	0.033	0.887
d 14 to 28	0.893	0.955	0.987	0.032	0.004	0.924	0.955	0.032	0.170	0.897	0.977	0.032	0.069
Overall	0.761	0.814	0.839	0.024	0.022	0.786	0.810	0.024	0.072	0.797	0.807	0.024	0.164

[a] Each value represents the least squares mean of eight pigs per treatment group over a 28-day period

[b] P-value represents linear effects of increased phosphorus via monosodium phosphate (MSP), turkey litter ash (TLA), or swine lagoon sludge (SLS) when added to the negative control diet (NC).

Table 3. Response of organ weights to supplemental P source

		MSP				TLA				SLS			
Organ weight, g	NC	+.075	+.15	SEM	P - value	+.075	+.15	SEM	P - value	+.075	+.15	SEM	P - value
Heart	280.72	257.45	267.38	9.076	0.218	268.89	261.08	9.076	0.188	274.43	275.90	9.076	0.694
Spleen	101.26	96.64	95.95	6.967	0.692	90.12	92.80	6.967	0.306	105.21	97.25	6.967	0.657
Liver, g	1154.88	1158.55	1225.99	46.68	0.330	1217.25	1116.44	46.68	0.611	1205.99	1137.31	46.68	0.741
Kidney, g	135.17	129.30	121.43	5.678	0.092	130.45	130.30	5.678	0.592	148.11	132.84	5.678	0.782
Organ weight, g/kg BW													
Heart	4.95	4.53	4.58	0.154	0.073	4.72	4.50	0.154	0.072	4.83	4.79	0.154	0.529
Spleen	1.78	1.70	1.64	0.125	0.531	1.58	1.6	0.125	0.238	1.85	1.68	0.125	0.498
Liver	20.33	20.37	20.93	0.751	0.559	21.30	19.26	0.751	0.358	21.15	19.75	0.751	0.482
Kidney	2.38	2.28	2.08	0.098	0.041	2.29	2.25	0.098	0.375	2.60	2.30	0.098	0.583

[a] Each value represents the least squares mean of eight pigs per treatment group over a 28-day period

[b] P-value represents linear effects of increased Phosphorus via monosodium phosphate (MSP), turkey litter ash (TLA), or swine lagoon sludge (SLS) when added to the negative control diet (NC).

Table 4. Response of serum chemistry to supplemental P source

		MSP				TLA				SLS			
	NC	+.075	+.15	*SEM*	*P - value*	+.075	+.15	*SEM*	*P- value*	+.075	+.15	*SEM*	*P - value*
Total Protein (g/dL)	6.05	6.04	6.28	0.143	0.205	6.29	5.86	0.143	0.277	6.238	6.238	0.143	0.418
Albumin (g/dL)	3.725	3.690	3.801	0.096	0.544	3.88	3.79	0.096	0.604	3.902	4.025	0.096	0.025
Globulin (g/dL)	2.325	2.350	2.480	0.102	0.316	2.41	2.08	0.102	0.101	2.338	2.213	0.102	0.484
A/G ratio	1.60	1.61	1.56	0.091	0.764	1.61	1.90	0.091	0.053	1.713	1.850	0.091	0.0600
P (mg/dL)	8.76	9.08	10.00	0.278	0.007	8.55	9.278	0.278	0.232	9.18	9.25	0.278	0.247
Ca (mg/dL)	13.66	13.10	12.41	0.226	.001	13.24	12.95	0.226	0.030	12.75	12.69	0.226	0.006
Mg (mEq/L)	2.41	2.33	2.25	0.095	0.291	2.26	2.33	0.095	0.342	2.29	2.28	0.095	0.313
K (mEq/L)	11.93	12.23	10.78	0.540	0.300	10.70	11.60	0.540	0.621	10.40	10.78	0.540	.052
Na (mEq/L)	144.75	144.00	147.38	1.567	0.177	144.25	146.75	1.567	0.239	147.88	146.75	1.567	0.371
NA/K ratio	12.38	12.38	13.86	0.587	0.185	13.63	12.75	0.587	0.621	14.38	13.88	0.587	0.023
Cl (mEq/L)	100.50	100.13	101.13	1.047	0.564	100.13	101.25	1.047	0.521	103.00	101.25	1.047	0.636
BUN (mg/dL)	15.13	14.86	15.13	0.838	1.000	137.50	14.38	0.838	0.480	13.50	13.63	0.838	0.214
Creatine (mg/dL)	1.13	1.19	1.29	0.059	0.028	1.15	1.18	0.059	0.586	1.11	1.18	0.059	0.541
AST(IU/L)	79.13	62.13	94.50	19.96	0.628	81.50	48.63	19.96	0.353	81.00	57.00	19.96	0.544
ALT (IU/L)	32.25	32.63	35.50	2.255	0.338	35.38	33.75	2.255	0.701	32.125	31.250	2.255	0.796
AP (IU/L)	250.75	282.63	325.88	28.22	0.073	310.50	280.88	28.22	0.545	289.13	260.13	28.22	0.778
GGT (IU/L)	131.75	94.50	126.38	30.41	0.869	193.63	113.00	30.41	0.601	137.88	174.00	30.41	0.417
Amylase (IU/L)	1360.6	1273.0	1471.13	159.55	0.579	1631.8	1366.3	159.55	0.978	1490.8	1660.5	159.55	0.229
CPK (IU/L)	6164.6	4225.3	7512.5	1965.5	0.554	7477.0	3166.8	1965.5	0.426	4142.9	3632.6	1965.5	0.291

Table 4 (continued)

		MSP				TLA				SLS			
	NC	+.075	+.15	*SEM*	*P - value*	+.075	+.15	*SEM*	*P-value*	+.075	+.15	*SEM*	*P - value*
PrecisionPSL (U/L)	8.500	8.50	9.25	0.681	0.399	9.13	8.00	0.681	0.454	9.63	8.50	0.681	1.000
WBC (10^3/uL)	12.41	9.81	12.91	1.404	0.812	9.98	13.18	1.404	0.669	12.75	11.08	1.404	0.508
RBC (10^6/uL)	7.41	7.91	8.05	0.274	0.132	7.70	7.70	0.274	0.971	7.86	7.94	0.274	0.264
HGB (g/dL)	13.51	14.20	14.18	0.366	0.224	14.05	13.59	0.366	0.879	14.39	14.68	0.366	0.089
HCT, %	43.8	45.50	46.00	1.31	0.237	45.25	44.00	1.309	0.942	46.88	47.38	1.309	0.137
MCV (fL)	59.50	57.75	57.50	0.890	0.125	59.00	59.38	0.890	0.912	59.50	60.13	0.890	0.588
MCH (pg)	18.30	18.03	17.66	0.339	0.190	18.31	18.39	0.339	0.840	18.39	18.56	0.339	0.562
MCHC (g/dL)	30.75	31.13	30.88	0.266	0.621	31.00	31.00	0.266	0.470	31.00	31.125	0.266	0.298
Platelet Count (10^3/uL)	219.00	183.50	183.50	47.99	0.657	221.00	245.00	47.99	0.552	173.25	259.75	47.99	0.529
Neutrophils (/uL)	3524.13	2403.50	3304.75	511.63	0.738	2806.13	3316.50	511.63	0.777	2292.38	3085.38	511.63	0.540
Lymphocytes (/uL)	8227.00	7008.00	8908.00	1015.5	0.657	6723.13	9198.88	1015.5	0.439	7426.38	10021.375	1015.5	0.609
Monocytes (/uL)	554.63	362.63	398.25	83.65	0.147	341.50	551.75	83.65	0.983	364.13	488.13	83.65	0.420
Glucose (mg/dL)	159.50	165.00	106.13	19.85	0.197	104.25	147.50	19.85	0.698	118.38	132.38	19.85	0.328
Amylase (IU/L)	1360.63	1273.00	1471.13	159.55	0.579	1631.75	1366.25	159.55	0.978	1490.75	1660.50	159.55	0.229

[a] Each value represents the least squares mean of eight pigs per treatment group over a 28-day period.

[b] P-value represents linear effects of increased Phosphorus via monosodium phosphate (MSP), turkey litter ash (TLA), or swine lagoon sludge (SLS) when added to the negative control diet (NC).

[c] Albumin/globulin ratio (A/G), phosphorus (P), calcium (Ca), magnesium (Mg), Potassium (K), sodium/potassium ratio (Na/K), chloride (Cl), blood urea nitrogen (BUN), Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase

Table 4 (continued)
(AP), gamma-glutamyl transferase (GGT), and creatine phosphokinase (CPK), precision pancreas-specific lipase (PrecisionPSL), white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC).

Table 5. Response of serum minerals to supplemental P source

		MSP				TLA				SLS			
mg/dL	**NC**	**+.075**	**+.15**	*SEM*	*P -value*	**+.075**	**+.15**	*SEM*	*P -value*	**+.075**	**+.15**	*SEM*	*P -value*
P	16.11	16.53	18.37	0.491	0.006	16.72	16.73	0.509	0.408	16.79	17.11	0.391	0.094
Ca	10.15	8.80	8.68	0.409	0.023	9.78	9.46	0.239	0.060	8.87	7.86	0.794	0.060
Mg	2.490	2.237	2.284	0.127	0.274	2.256	2.325	0.094	0.233	2.215	2.128	0.098	0.021
Fe	0.235	0.251	0.241	0.024	0.872	0.265	0.247	0.026	0.762	0.253	0.237	0.032	0.976
Cu	0.209	0.208	0.200	0.007	0.386	0.205	0.205	0.010	0.764	0.227	0.207	0.014	0.898
Zn	0.099	0.111	0.127	0.016	0.224	0.120	0.116	0.011	0.274	0.135	0.113	0.014	0.467

[a] Each value represents the least squares mean of eight pigs per treatment group over a 28-day period
[b] P-value represents linear effects of increased Phosphorus via monosodium phosphate (MSP), turkey litter ash (TLA), or swine lagoon sludge (SLS) when added to the negative control diet (NC).
[c] Phosphorus (P), calcium (Ca), Manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn)

Table 6. Response of bone strength and bone composition to supplemental P source

		MSP				TLA				SLS			
	NC	**+.075**	**+.15**	*SEM*	*P - Value*	**+.075**	**+.15**	*SEM*	*P - Value*	**+.075**	**+.15**	*SEM*	*P - Value*
Peak Breaking Strength (kg)	65.527	72.005	84.099	4.2719	0.0082	64.947	81.055	3.9619	0.0150	71.494	77.184	4.2919	0.077
Length (mm)	65.275	64.600	63.788	1.0393	0.329	65.275	65.325	0.8138	0.966	66.574	66.488	0.9596	0.396
Ash Wt (g)	3.6225	3.8463	4.0363	0.1134	0.022	3.8600	4.0525	0.0913	0.005	3.9286	4.0463	0.1074	0.015
Ash Wt (%)	40.2764	40.9806	43.6208	1.2639	0.082	41.7053	42.4560	1.3937	0.287	41.2447	41.5759	0.9979	0.373
Total P (g)	0.1631	0.1694	0.1837	0.0053	0.017	0.18016	0.18024	0.0058	0.057	0.1686	0.1917	0.0082	0.028
Bone P (%)	4.4948	4.4140	4.5527	0.0776	0.607	4.6725	4.4432	0.1244	0.773	4.2495	4.7013	0.1297	0.279
Total Ca (g)	0.3344	0.3442	0.3682	0.0112	0.052	0.3637	0.3628	0.0120	0.118	0.3409	0.3866	0.0170	0.048
Bone Ca (%)	9.2203	8.9616	9.1234	0.1526	0.660	9.4406	9.9450	0.2666	0.477	8.6006	9.5038	0.2736	0.476
Ca/P	2.053	2.031	2.005	0.0104	0.006	2.02	2.013	0.0116	0.031	2.024	2.024	0.0089	0.039

[a] Each value represents the least squares mean of eight pigs per treatment group over a 28-day period

[b] P-value represents linear effects of increased Phosphorus via monosodium phosphate (MSP), turkey litter ash (TLA), or swine lagoon sludge (SLS) when added to the negative control diet (NC).

Table 7. Response of apparent total track digestibility of P, Ca, Mg, Fe, Cu, Zn

		MSP				TLA				SLS			
(%)	**NC**	**+.075**	**+.15**	*SEM*	*p-Value*	**+.075**	**+.15**	*SEM*	*p-Value*	**+.075**	**+.15**	*SEM*	*p-Value*
P	37.407	48.142	42.345	1.443	0.038	42.975	40.510	1.443	0.256	46.070	43.776	1.443	.002
Ca	69.587	71.790	69.962	1.326	0.869	72.367	68.990	1.326	0.811	73.549	74.139	1.326	0.811
Mn	7.558	-1.908	-8.176	2.246	< 0.001	1.852	-5.904	2.246	0.003	5.358	0.504	2.246	0.009
Fe	-12.110	0.659	-6.431	6.585	0.620	2.436	6.563	6.585	0.108	3.942	-6.667	6.585	0.686
Cu	-0.375	-3.774	18.174	2.254	< 0.001	9.834	11.517	2.254	0.004	8.655	2.803	2.254	0.088
Zn	-27.140	-18.502	-25.743	3.540	0.798	-23.329	-17.232	3.540	0.055	-5.199	-6.132	3.540	0.002

[a] Each value represents the least squares mean of eight pigs per treatment group over a 28-day period
[b] P-value represents linear effects of increased Phosphorus via monosodium phosphate (MSP), turkey litter ash (TLA), or swine lagoon sludge (SLS) when added to the negative control diet (NC).
[c] Phosphorus (P), calcium (Ca), Manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn)

Table 8. Response of ADG, serum P, peak breaking force, bone ash, bone P and apparent total tract digestible P, to supplemental P and source

	Slope			Relative Bioavailability (%)	
	MSP	TLA	SLS	TLA	SLS
ADG, kg/d	0.5583x	0.3274x	0.3632x*	58.64	65.05*
Serum P, mg/dL (serum chemistry)	8.2467x	3.4133x*	4.3267x*	41.39*	52.47*
Serum P, mg/dL (mineral analysis)	13.12x	4.8907x	7.096x	37.28*	54.09
Peak Breaking Force	116.29x	85.707x	78.027x	73.75	67.1
Bone Ash, %	19.715x	14.531x*	8.6635x*	73.71*	43.94*
Total Bone P (g)	0.1267x	0.1369x	0.1672x	108	131.97
Bone P (%)	0.0933x*	0.1987x*	.4472x*	212.97*	479.31*
Fecal P Digestibility, %	54.97x	31.401x*	56.791x	57.12*	103.31

*No Statistical Significance or Tendency (P>0.1)

Figure 1. Regression Analysis of Average Daily Gain as a function of P source

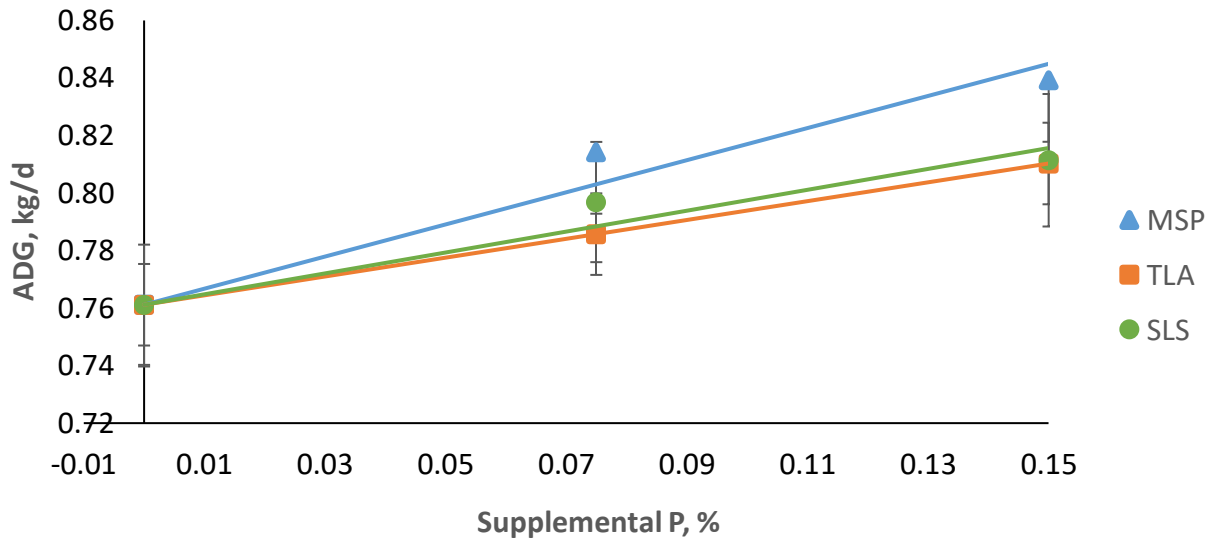

Figure 2. Regression Analysis of Antech Serum P as a function of P source

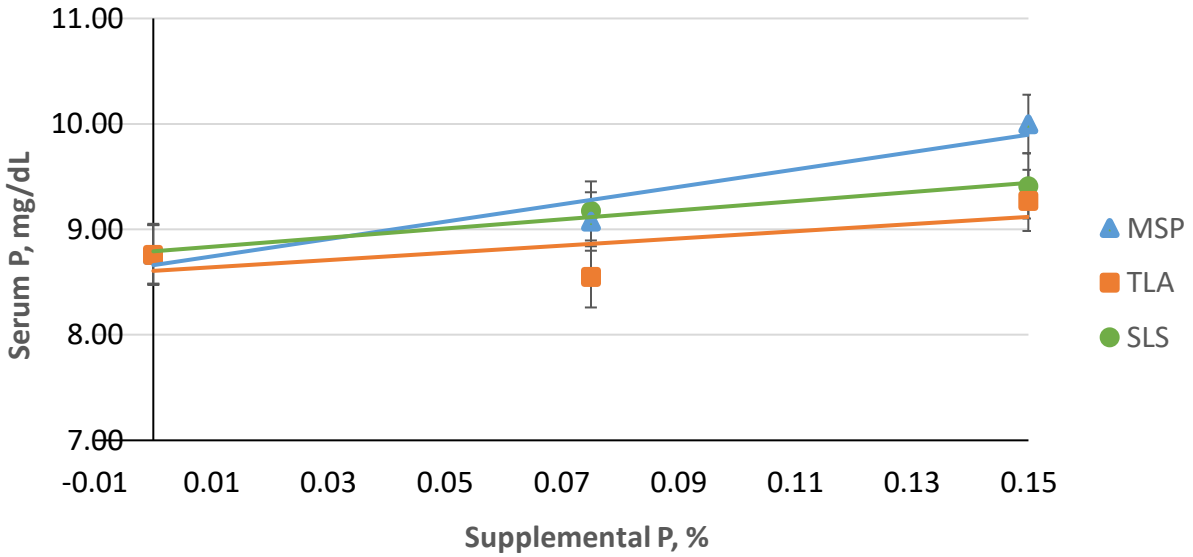

Figure 3. Regression Analysis of EATS Serum P as a function of P source

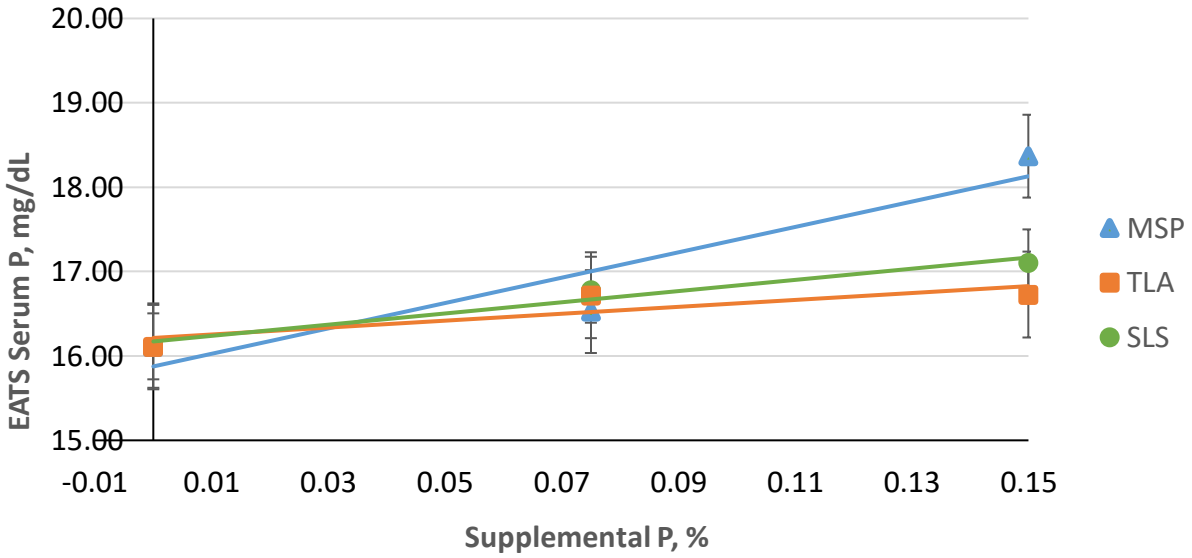

Figure 4. Regression Analysis of Peak Breaking Force as a function of P source

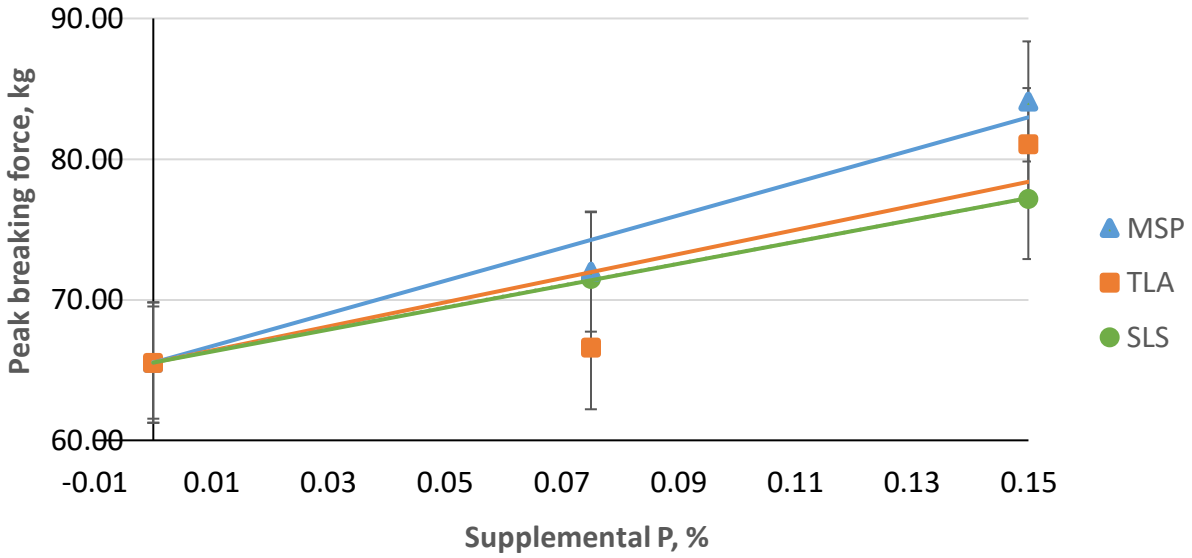

Figure 5. Regression Analysis of Bone Ash Percentage as a function of P source

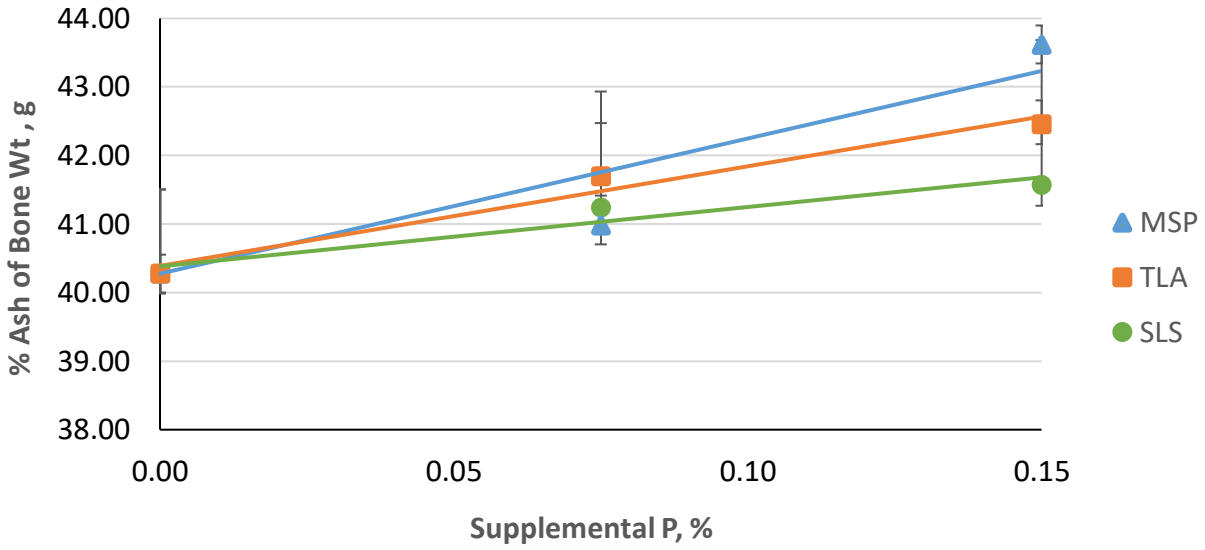

Figure 6. Regression Analysis of Total Bone P as a function of P source

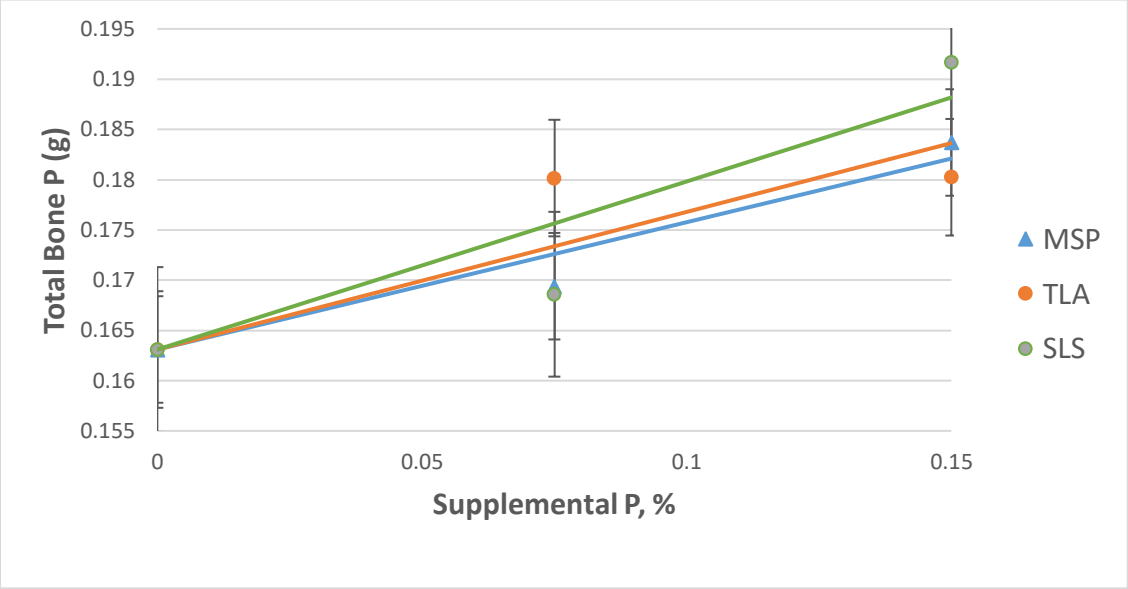

Figure 7. Regression Analysis of Apparent total tract Digestibility of P