ABSTRACT

SOLER DIAZ, FERNANDO DAVID. Evaluation of Ashed and Dried Swine Lagoon Sludge as a Potential Alternative Dietary Phosphorus Source for Pigs. (Under the direction of Dr. Eric van Heugten).

Excess phosphorus (P) excretion in manure can have negative environmental consequences but could be recycled into the diet as a valuable nutrient. Therefore, this study evaluated the potential of feeding recycled ashed swine lagoon sludge (ASLS) as an alternative P source for swine. In experiment 1, 56 individually housed pigs (body weight of 44.14±4.11 kg) were blocked by sex and initial body weight and randomly assigned to 7 dietary treatments (8 blocks/treatment). Treatments included a negative control diet (NC; 0.45% Ca and 0.12% available P representing 40% of the requirement), and the NC supplemented with either monosodium phosphate (MSP) or ASLS to provide an additional 0.05%, 0.10%, and 0.15% of total P, while maintaining a constant Ca:P ratio of 1.1:1. Ash was produced through combustion reaching a peak temperature of 1,800°C. Pigs were limit-fed at 3 times maintenance requirements based on average body weight for each block. Feed was provided twice daily for the 35-day study. Diet analysis showed that the NC diet exceeded targeted Ca and P concentrations and was excluded from statistical analysis. Thus, data were analyzed as a 2×3 factorial arrangement with source (MSP or ASLS) and level (0.05, 0.10, and 0.15%) as factors. Final BW and overall average daily gain were not affected by treatments ($P>0.27$). Serum P concentrations linearly increased ($P=0.022$) in pigs supplemented with MSP and tended to increase with ASLS ($P=0.098$). Slope-ratio analysis for serum P indicated a bioavailability of P of 68.89% for ASLS. Analyzed P concentrations (DM-basis) in feces increased linearly with the addition of P from MSP ($P<0.001$) and ASLS ($P=0.014$), with no difference between sources ($P=0.809$). Apparent total tract digestibility of P increased ($P<0.001$) with added P for both MSP and ASLS with no difference between sources ($P=0.722$). Third metacarpal bone breaking force increased linearly with increasing P from MSP (P<0.001), but not from ASLS (P=0.695). Results indicate that ASLS has lower bioavailability compared to MSP as the standard but can be used as an alternative dietary P source if approved as a feed ingredient. In experiment 2, the practical effect of feeding dried swine lagoon sludge (DSLS) and ASLS as a P supplement compared to monocalcium phosphate (MCP) on growth performance, feed efficiency, serum P, and apparent total tract digestibility of P was evaluated in nursery pigs. Weaned pigs (n=192; 28-day old; BW=6.99± 0.79 kg) were randomly assigned within sex and body weight blocks to 1 of 3 dietary treatments using 48 pens (4 pigs/pen; 16 blocks/treatment). Dietary treatments included: a negative control (NC) diet with MCP as the main P source, a diet with ASLS as the phosphorus source, and a diet with DSLS as the P source. Diets contained 80% of the estimated P requirement. At the end of the 28-day study, pigs fed the ASLS diet had a lower body weight (P< 0.001; 20.46 kg) than pigs fed MCP(21.77 kg) and DSLS(21.77 kg) diets, while feed intake was higher for DSLS(P=0.002) compared to MCP and ASLS diets. Serum P measured on day 14 tended to be higher in pigs fed the DSLS (P=0.06) compared to ASLS but not to the MCP diet. On day 28, serum P was lower (P<0.001) in pigs fed ASLS compared to MCP and DSLS diets. The preliminary economic assessment estimated the net cost of P recycled from DSLS is 1.45 times more expensive than the cost of P provided by MCP. Since DSLS serves as an input for the ASLS process, the cost of P from ASLS is expected to be even higher. However, this estimate is influenced by factors such as the specific management options used for sludge.

Evaluation of Ashed and Dried Swine Lagoon Sludge as a Potential Alternative Dietary Phosphorus Source for Swine.

by
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BIOGRAPHY

Fernando Soler was born on May 16, 2002, in the capital of Honduras, Tegucigalpa, Francisco Morazán. Fernando spent his formative years in a small town in Comayagua, Honduras, called San Luis. Growing up in a family deeply rooted in coffee cultivation—his father being a second-generation coffee grower—he was immersed in agriculture from a young age. After the tragic loss of his father during childhood, Fernando's mother worked tirelessly to fulfill his father's dream of seeing him pursue higher education at Zamorano University, a prestigious agricultural institution in Latin America located in Honduras.

During his time at Zamorano, Fernando developed a keen interest in swine production through hands-on modules and coursework, which inspired him to focus on this field. In his senior year in 2022, he secured an internship for one year with Smithfield, a leading hog production company in the United States at the Missouri Division. This experience deepened his understanding of swine nutrition and sparked a desire to further his education through advanced studies.

In 2023, Fernando began pursuing a master's degree in Animal Science and Nutrition with a minor in statistics, primary focus in swine nutrition at North Carolina State University under the mentorship of Dr. Eric van Heugten. Now, upon graduation, Fernando has decided to continue his academic journey at North Carolina State University, where he will pursue his Ph.D. under Dr. van Heugten's guidance once again.

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CHAPTER I: Literature Review

Introduction

Global pork production is led by China, followed by the European Union and the United States. In 2022, the United States produced over 12 million metric tons of pork (Shahbandeh, 2024a). The pork industry plays a crucial role in providing a significant economic value and contributing majorly to the overall U.S. economy. This industry not only supports countless jobs and livelihoods but also drives advancements in agricultural practices and technologies (Cook & Schulz, 2024). The importance of the pork industry extends beyond economic metrics, as it also encompasses food security and nutritional benefits, serving as an essential protein source for consumers offering a reliable and nutritious food source to millions of people around the world. Pork production in the United States has experienced consistent growth over the past several years, reflecting its importance as a key agricultural industry. In 2023, the nation produced approximately 27 billion pounds of pork, underscoring the scale of the industry and its contribution to both domestic consumption and international markets (Shahbandeh, 2024b). Pork production in the United States is dominated by Iowa, which, as of March 2024, reported an inventory of approximately 25.1 million hogs and pigs. Minnesota and North Carolina follow Iowa as the second and third largest producers, respectively, highlighting their significant roles in the nation's pork industry (USDA, 2024).

Cost management

Numerous factors influence the swine production chain, but feed cost is particularly significant, accounting for approximately 60-70% of total production expenses (Johnston, 2018). As such, feed costs represent a major area of concern and opportunity within the industry with effective management of total hog production costs requiring management of feed costs. With feed expenses comprising a substantial share of overall production costs, there is immense pressure on producers to find cost-effective solutions without compromising the nutritional value essential for animal health and productivity (Gilliam, 2020). Reducing these costs can significantly enhance profitability, making it essential to explore various strategies for optimizing diet formulation. This includes evaluating alternative feed ingredients, improving nutrient utilization, and enhancing feed efficiency. Reliance on external sources for minerals presents a chance to reduce costs by finding and using alternative sources (Lammers et al., 2008). Identifying cost-effective alternatives for swine diets not only provides substantial financial benefits but also contributes to the sustainability of swine production by potentially reducing dependency on conventional feed resources. Therefore, a comprehensive and precise approach to diet formulation, focusing on both economic and nutritional aspects, is crucial for the long-term success and viability of the swine industry. One of the key components in swine diets is phosphorus (P), a crucial macro-mineral with many essential functions. Phosphorus is the second most abundant following calcium and it is the third most expensive ingredient in swine diets (Cromwell, 2015).

Phosphorus

Providing calcium (Ca) and phosphorus (P) in the right proportions at the right place is vital for the mineralization of bones and teeth, ensuring proper skeletal development and strength (Kumar et al., 2020). Skeletal mineralization begins with matrix vesicles derived from the plasma membranes of osteoblasts and chondrocytes (Bottini et al., 2017). These specialized vesicles play a crucial role in bone formation by hydrolyzing phosphoric esters to produce inorganic orthophosphates. The generated orthophosphates are then actively transported into the matrix vesicles through specific cotransporters. Within the vesicles, these orthophosphates undergo a series of biochemical processes to form hydroxyapatite, a naturally occurring mineral form of calcium apatite. Hydroxyapatite is a major component of normal bone and teeth, contributing significantly to their structural integrity and comprising about 70% of bone and 90% of tooth enamel (Michigami & Ozono, 2019). Beyond its structural role, P is integral to the metabolism of carbohydrates and fats, facilitating energy production and utilization within the body. Phosphorus is a fundamental component of adenosine triphosphate (ATP), the primary molecule that stores and transfers energy in cells. Energy is essential for all the processes that sustain life within an organism (Boyer, 1997). Many of these processes occur continuously, such as the metabolism of food, the synthesis of large biologically important molecules like proteins and DNA, and the transport of molecules and ions throughout the body. It is also necessary for the synthesis of proteins as a cofactor (Chu et al., 2022), which are crucial for the growth, maintenance, and repair of cells and tissues. Given these diverse and critical functions, ensuring adequate phosphorus levels in swine diets is essential for promoting optimal growth, health, productivity and thereby generating financial benefits.

Phosphorus in swine

Most of the P absorption in swine occurs in the upper gastrointestinal tract, primarily in the proximal duodenum, where it is absorbed in its inorganic form. Nevertheless, the efficiency of P absorption can be significantly influenced by the dietary Ca/P ratio (Williams et al., 2023). High concentrations of Ca can reduce P absorption by forming insoluble tricalcium phosphate in the intestinal tract, making P unavailable for absorption. Additionally, Vitamin D also plays a vital role in the absorption and metabolism of both Ca and P. It regulates the synthesis of transport proteins in the intestine that are essential for the uptake of these minerals. Without adequate levels of vitamin D, the efficiency of Ca and P absorption is compromised, leading to potential deficiencies even if the dietary intake of these minerals is sufficient (Cromwell, 2015).

Factors such as the source of P, the presence of phytates (a molecule that contains P but is largely indigestible by pigs due to their lack of enzyme phytase to break it down), and the overall diet composition must be considered to optimize P nutrition in swine (Humer et al., 2015) . By controlling these factors, it is possible to enhance phosphorus absorption and utilization, promoting better growth, skeleton development, and overall health in swine. The P is essential for pigs as it supports various biological functions, primarily aiding in skeletal structure, which functions to maintain body shape, protect internal organs, and serve as levers for movement. A total of 99% of the body's calcium is located in the skeleton, with the remaining 1% distributed among the blood, muscles, organs, and other soft tissues. In a similar pattern, 75 to 80% of the body's phosphorus is found in the skeleton, while the remaining 20 to 25% resides in soft tissues. Within skeletal tissue, Ca and P are initially deposited as tricalcium phosphate, which subsequently transforms into hydroxyapatite. The Ca:P ratio in bone is consistently maintained at 2.1:1, even in cases of severe deficiencies of one or both minerals (Becker et al., 2020). This underscores the body's tight regulation of these essential elements to preserve bone structure and function (Cromwell, 1976).

The mobilization of Ca and P within the body is intricately regulated by a hormonal system involving parathyroid hormone (PTH), fibroblast growth factor (FGF23), calcitonin, and vitamin D (Khan et al., 2022). Phosphorus primarily exists and is absorbed in its structural ionic form, PO_4^{3-}, which plays a crucial role as a constituent of hydroxyapatite, the mineral foundation of the skeleton, and serves as the backbone of DNA at the molecular level (Šponer et al., 2012). PTH and FGF23 are the primary regulators of phosphate balance. PTH inhibits phosphate reabsorption by activating protein kinase A (PKA) and protein kinase C (PKC) signaling pathways, which lead to the downregulation of NaPi-IIa and NaPi-IIc transporters responsible for phosphate reabsorption in the proximal tubule, reducing phosphate uptake by the kidneys (Carpenter, 2022). FGF23 inhibits renal tubular reabsorption of phosphate but, it uses mechanisms that are independent of PTH. The FGF23 acts via extracellular signal-regulated kinases 1 and 2 (ERK1/2) and the co-receptor α-klotho for activation while it also decreases vitamin D synthesis (Leung & Crook, 2019). PTH is crucial in regulating Ca levels in the body and facilitates the mobilization of calcium by stimulating its release from bone reserves. It also enhances Ca absorption in the small intestine. Additionally, PTH influences renal 1α-hydroxylase (CYP27B1), the enzyme responsible for converting calcidiol, the storage form of vitamin D, into its active form, calcitriol (Bikle, 2021a). Calcitriol, produced in the kidneys, further increases the absorption of minerals in the intestine, thus maintaining optimal Ca balance and supporting overall mineral homeostasis (Brenza et al., 1998). Thyrocalcitonin, commonly referred to as calcitonin, is produced by the thyroid gland and acts to lower blood calcium levels by inhibiting bone resorption and increasing calcium excretion by the kidneys (Foster, 1968). Vitamin D, known as the sunshine vitamin, is essential for the effective absorption of Ca and P from the intestine and for their proper deposition in bones Vitamin D, whether obtained from sunlight or dietary sources, is first converted in the liver to 25-hydroxyvitamin D_3 (Bikle, 2021b). When released into the body, 25-hydroxyvitamin D_3 is converted in the mitochondria by the enzyme 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) into its active form, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). This active form of vitamin D is crucial for maintaining the balance of Ca and P in the body (Holick, 2006). Together, these hormones maintain a delicate balance of calcium and phosphorus, ensuring optimal bone health and metabolic function.

Phosphorus sources

Dietary P is mainly sourced from plant and animal feedstuffs, as well as from inorganic phosphorus sources (Waldroup, 1999). These ingredients collectively contribute to the P intake necessary for the physiological functions in the pigs. The P in plant sources is predominantly stored as phytic acid, which constitutes up to 80% of the P content found in seeds (Bohn et al., 2008). Phytic acid can contribute significantly to the seed's dry weight, reaching concentrations as high as 1.5%. Phytic acid plays a crucial role in plants by strongly binding to positively charged metallic cations like calcium, iron, potassium, magnesium, manganese, and zinc (Ribeiro et al., 2019). Phytic acid serves as a major reservoir of P in plants. It releases the required energy and minerals for germination; it is also involved in stress tolerance and defense mechanisms by preventing oxidation of mineral cations (Pramitha et al., 2021). Its chelation ability ensures the plant retains important minerals and protects them from being wasted. The capacity of phytic acid to strongly complex these minerals makes them insoluble and unavailable for absorption in monogastrics, impacting their bioavailability as essential nutrients (Bohn et al., 2008). Phytic acid is recognized as an anti-nutritional factor in swine nutrition, significantly impairing the digestibility of P, energy, and essential nutrients crucial for pigs (Woyengo, 2022). Its detrimental effect on P availability arises from the fact that pigs lack the necessary enzyme to break down the phytate molecule to release P (Gebhardt et al., 2021). Therefore, promoting the breakdown of phytate in the gastrointestinal tract is essential to enhance phosphorus utilization and mitigate the anti-nutritional effects linked to phytate. Phytases are enzymes that catalyze the breakdown of phytate, releasing P from the phytate molecule (Rizwanuddin et al., 2023). These enzymes can be sourced exogenously, primarily from bacterial and fungal fermentation. These phytases can be divided into 3-and 6 phytases, depending on the carbon site for hydrolysis of phytic acid (Dersjant-Li et al., 2015). The first commercialized phytase as feed additive was fungal phytase from *Aspergillus niger* (Maulana et al., 2023). Supplementing swine diets with phytase enzymes significantly increases the availability of phytate-bound phosphorus, thereby reducing the need for inorganic phosphorus supplements. Besides improvements in nutrient utilization, phytase supplementation also decreases the amount of undigestible P excreted in manure, thus contributing to environmental sustainability (Brumm, 2001). The P derived from animal-based feedstuffs, such as meat, fish, and bone meal, is not only abundant but also has significantly higher digestibility compared to plant-based sources. These animal-derived feeds provide a more readily available form of P, enhancing its absorption and utilization by swine (Jongbloed & Kemme, 1990).

Bioavailability and digestibility

In animal nutrition, the term "bioavailability" refers to the proportion of a digested nutrient that is utilized by the animal's body for its physiological function. One of the most commonly used designs in animal nutrition for measuring mineral bioavailability is the slope ratio assay (Byrne & Murphy, 2022). This method provides information on the ability of a nutrient in a feed ingredient to be utilized compared to a standard nutrient source with an expected known availability. In the case of P, monosodium phosphate, monocalcium phosphate, and dicalcium phosphate have been used as the standard P source with known bioavailability (Zhai et al., 2022). Although bioavailability cannot be measured in absolute terms, this method allows for a quantifiable comparison, highlighting the relative effectiveness of various mineral sources in supporting the animal's metabolic processes (Littell et al., 1997). The bioavailability of a test mineral source relative to a standard source is estimated by comparing the slopes of their respective response curves. Specifically, values of the independent and dependent variables, x and y, are determined for each experimental unit to construct a regression line, using increasing levels of the nutrient of interest from both the test and standard sources, ensuring equal levels across all sources. To estimate the bioavailability of a test mineral source, the slope of the regression line for the test source is divided by the slope of the regression line for the standard source. These slopes are determined by the response to increasing levels of the nutrient of interest, with the response being the biological effect or outcome used as criteria. These slopes originate from a common intercept, representing the baseline condition where no supplementation of the nutrient is provided, and this outcome will depend on the mineral of interest (Figure 1). In the case of P, bone-breaking strength and bone ash percentage measurements were found to be the most suitable criteria for slope ratio assays (Soares, 1995). This ratio reflects the amount of the test mineral required to produce an effect equivalent to that produced by a given amount of the standard mineral. By comparing these slopes, researchers can evaluate the relative efficiency and effectiveness of the test mineral in being absorbed and utilized by the animal's body. This comparative measure is important in animal nutrition as it helps determine the appropriate dosage and efficacy of various feed ingredients, ensuring that the animals receive optimal nutrition (Littell et al., 1995).

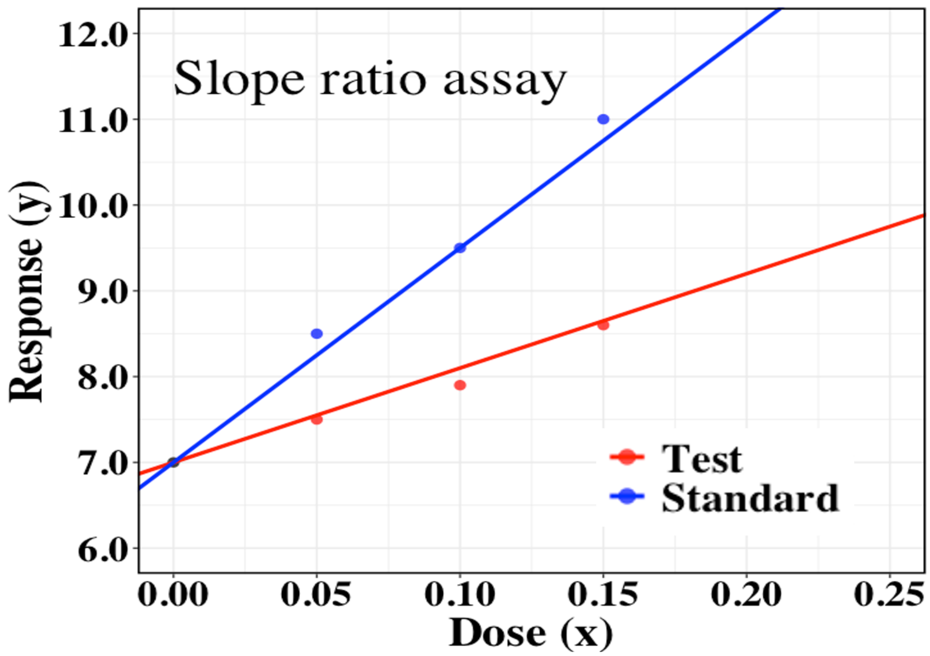


Figure 1. Slope ratio assay procedure

Other methods to determine the nutritional value of phosphorus in dietary ingredients and swine feeds include apparent total tract digestibility (ATTD), standardized total tract digestibility (STTD), and true total tract digestibility (TTTD)(Zhai et al., 2022). Digestibility of phosphorus is the proportion of phosphorus absorbed by the digestive system of the animal, measured by the difference between total dietary P intake and total fecal P excretion expressed relative to the total phosphorus intake (NRC, 2012).

$$ATTD\ of\ P(\%) = \frac{(P\ intake - P\ excreted)}{P\ intake} \times\ 100$$

This approach requires the measurement of feed and phosphorus intake and the total collection of feces and associated phosphorus excretion, which can be both expensive economically and time-consuming (Battelli et al., 2020). Therefore, the use of indigestible markers such as chromic oxide or titanium dioxide is often employed to estimate ATTD through a dilution coefficient, providing a more efficient and practical method (Schalla et al., 2012).

$$ATTD\ of\ P(\%) = \left[1 - \left(\frac{\%\ marker\ in\ feed}{\%\ marker\ in\ feces} \times \frac{\%\ P\ in\ feces}{\%\ P\ in\ feed}\right)\right] \times\ 100$$

Calculations of digestibility by using the ratio of an indigestible marker in feed and feces would allow a simple collection of a representative sample of feces instead of the total collection (Sales, 2012).

Standardized total tract digestibility (STTD) is a more current and also widely used measurement for assessing the digestibility of nutrients, including minerals. Unlike apparent total tract digestibility (ATTD), STTD provides a more accurate measure of mineral availability by accounting for endogenous losses. Basal endogenous losses refer to losses within the body due to physiological, metabolic, and digestive processes, independent of dietary intake. Endogenous P losses are excreted from saliva and intestinal cells as well as pancreatic and bile secretions (She et al., 2017a). The use of STTD instead of ATTD in animal diet formulations offers a more precise reflection of the true availability of nutrients from the diets. While ATTD simply measures the apparent absorption of nutrients, which include both dietary and endogenous components, STTD standardizes for these endogenous losses. When P excretion is measured in feces using the ATTD method, both dietary P intake and endogenous losses contribute to the fecal output, leading to a lower digestibility value. However, when endogenous losses are accounted for in the STTD method, the resulting digestibility value is higher (She et al., 2017b). This difference is crucial, as it helps to avoid overestimation of nutrient availability based on dietary intake alone. Recent literature has highlighted the advantages of formulating diets based on STTD because the values of nutrients are additive when combining ingredients in mixed diets (Gonçalves et al., 2016; Stein, 2024; Zhai et al., 2022). This allows for better predictions of overall nutrient availability in complete diets and improves the precision of diet formulation to meet the nutritional needs of animals while minimizing waste or excess (Stein, 2024).

Standardized total tract digestibility can be calculated using the following equation(Almeida & Stein, 2010):

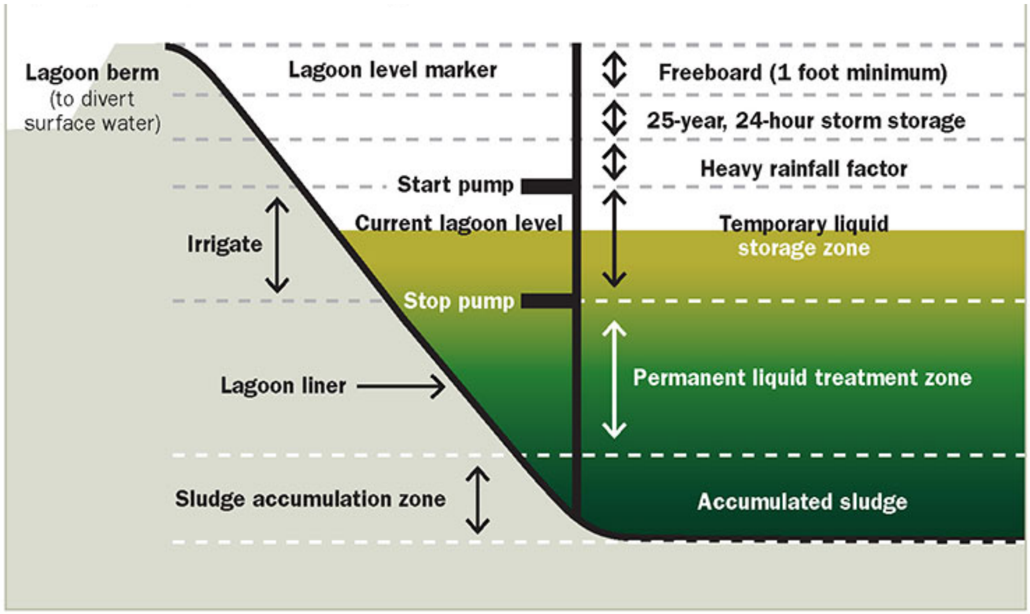
$$STTD\ (\%) = ATTD - \left(\frac{Endogeneous\ P\ Losses}{P\ intake}\right) \times 100$$

Several studies have been conducted using P-free diets to estimate basal EPL for STTD measurements (Petersen & Stein, 2006). A mean expected value of 190 mg/kg DMI (NRC, 2012) has been suggested.

The true total tract digestibility of P can be calculated with a linear regression method similar to Pettey et al. (2006) to evaluate the P absorbed by the pigs relative to its total P intake while accounting for all endogenous losses. Unlike STTD, which only adjust for "basal endogenous losses" a term used to refer to endogenous losses independent of feed ingredients, TTTD considers both basal endogenous losses and diet-induced (specific) endogenous losses (Ammerman, 1995). A key component of the use of this method is to establish a linear relationship between the apparent digestible intake and total intake of the P in diets (She et al., 2017a). However, it has been suggested by Dilger and Adeola (2006) that the estimation of endogenous losses by linear regression is highly variable, therefore, effects are difficult to detect. The most commonly used method to formulate diets and estimate P digestibility is the STTD. More accurate digestibility values of P sources in swine nutrition can help in meeting the phosphorus requirements more effectively while minimizing environmental impact due to excretion of excess P and potential economic savings.

Sludge from swine lagoons

Swine lagoon sludge is the buildup of inorganic material that occurs during normal lagoon operations over time. Swine lagoons are structures designed for the storage and treatment of swine manure by utilizing anaerobic bacteria to decompose organic matter in manure (P. Patil et al., 2023). While they reduce manure solids, the anaerobic treatment leads to slow accumulation and settling of an inorganic residue rich in nutrients (Sharara & Owusu-Twum, 2020). Accumulation of sludge reduces the effectiveness of the lagoon; therefore, periodic removal of sludge is essential for treating animal waste to avoid the reduction of the lagoon volume treatment (Figure 2). Excessive sludge accumulation can result in non-compliance with regulatory permit requirements. Effective lagoon management requires adherence to specific operational, monitoring, and reporting protocols, including proper land application practices, maintaining acceptable waste levels, timely sludge removal, regular inspection of lagoon structures, necessary repairs, and conducting soil sampling prior to land application (NCEQ, 2020).



From USDA-NRCS Agricultural Waste Management Field Handbook. 2009

Figure 2. A single-stage anaerobic manure treatment lagoon

The recommended treatment volume size of the lagoon depends on the daily loading rate of volatile solid and mean temperature of the region (Owusu-Twum & Sharara, 2020). Lagoon sludge contains high concentrations of various minerals, including Ca, P, K, Mg, S, Na, Zn, Mn, Fe, and Cu. Of greatest interest is P because it is the third most expensive nutrient in swine diets and would provide the greatest financial benefit. Swine manure sludge contains approximately 3.83 g/L of P (mean of samples obtained from 27 lagoons) and 9.02 % total solids (25 lagoons), which is approximately 4 to 5% of P on a dry matter basis (Sharara & Owusu-Twum, 2020). The nutrient content in sludge depends on multiple factors such as the age of the animals, temperature, methods to collect and store, and moisture content (Chastain et al., 1998). Effluent from lagoons is commonly used as fertilizer for crops integrating animal production, lagoon treatment, and nutrient recycling, while lagoon sludge presents a separate challenge. Over time, sludge accumulates at the bottom of lagoons, concentrating nutrients and potentially leading to management and environmental issues if not properly addressed. One of the primary issues associated with pork production and its expansion is the disposal of the animal manure and control of odor emission associated with the management (Hatfield et al., 1998). Improper management of sludge can lead to significant environmental and health issues, including the infiltration of excessive mineral concentrations into water sources. This contamination can harm local ecosystems and pose serious risks to human and animal health. Consequently, the development of innovative sludge management alternatives has become increasingly important for swine farmers. Effective sludge management not only helps mitigate environmental risks but also promotes sustainability in agricultural practices. Before removing sludge from lagoons, it is highly recommended that producers collect representative sludge samples to analyze nutrient concentrations. This analysis allows farmers to determine the best utilization strategy for this resource, whether for direct application as fertilizer, composting, or other emerging alternatives (Sharara & Kulesza, 2020). Various pathways have been developed to manage swine lagoon sludge (Figure 3), aiming to remove it efficiently and transform it into valuable by-products that enable the recycling of its nutrient content. These pathways focus on optimizing the recovery of essential nutrients, such as P, while addressing environmental concerns and operational challenges associated with sludge management. By converting sludge into usable products, these approaches contribute to more sustainable and resource-efficient agricultural practices.

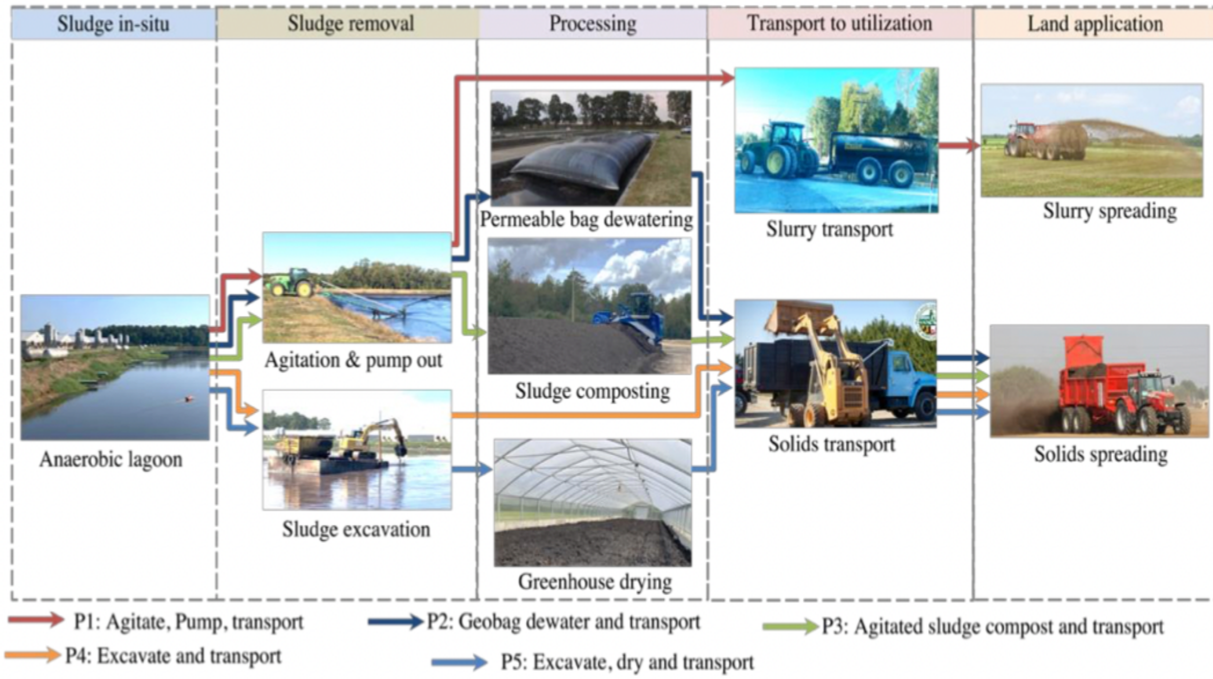


Figure 3. Process flow diagram of sludge utilization pathways (Patil, 2023)

Recent research has highlighted the feasibility of co-composting swine sludge with locally available materials, such as poultry litter. This method stabilizes the sludge by reducing moisture, accelerating the decomposition of organic material, and decreasing pathogen levels, but also enhances its nutrient profile, making it a more effective soil amendment (Patil, 2023). Composting is particularly advantageous for areas where land availability for direct sludge application is limited. By transforming sludge into compost, farmers can create a stable, odorless product with an easier transport and application to fields (Patil et al., 2023). Direct application of sludge as fertilizer remains one of the most common practices among swine farmers. This approach improves soil fertility and nutrient efficiency, retroactively recycling nutrients back into the soil (Bertici et al., 2022). However, it presents several challenges, including land availability constraints, potential runoff, and the need for precise application to avoid overloading soil with nutrients if not appropriately managed. Such limitations have prompted producers and technology developers to explore innovative solutions that extend beyond traditional land application methods. These emerging approaches aim to address both environmental concerns and practical challenges faced by farmers while enhancing the economic viability of sludge management (Sharara & Kulesza, 2020).

Recently, innovative ideas have emerged to recycle nutrients from sludge and reintroduce them into the production chain by incorporating sludge as a mineral source in swine diets. This concept has gained attention as a sustainable strategy to reduce dependency on traditional mineral supplements while managing sludge in an environmentally responsible way. New approaches including drying and ashing sludge have been explored to repurpose swine sludge as a potential ingredient in animal feed rather than relying solely on its application as a fertilizer in fields. This approach not only eliminates the risk of mineral overapplication to the soil but also enables the recovery and efficient utilization of essential nutrients, such as P. By integrating these nutrients back into the agricultural production cycle, these innovative methods contribute to more sustainable nutrient management and reduce environmental impacts associated with traditional sludge disposal practices (See et al., 2004). A recent study conducted by McAuley (2022) evaluated dried swine lagoon sludge as a P source in swine diets, reporting bioavailability values of 67% and 72% for the P in dried sludge. These findings demonstrate the potential of dried sludge to provide a substantial portion of the dietary phosphorus needs of swine. Additionally, the concept of ashing sludge has gained traction as an innovative processing method. By burning off the organic material, this approach effectively eliminates potential pathogens and bacteria while significantly reducing the volume of the sludge. The resulting ash is a more nutrient-dense product that is easier to transport and has potential use as a mineral supplement, providing a practical and efficient solution for nutrient recovery and management (See et al., 2004). Previous research by van Heugten et al. (2006) explored the digestibility of ashed swine manure solids, comparing different combustion approaches to understand their effects on nutrient availability. The study assessed the effectiveness of two gasification technologies, the Brookes Gasification Process (BGP) gasifier and the Bud Klepper Technologies (BKT) gasifier, for combusting swine manure solids to produce ash as a phosphorus supplement (Table 1 and 2).

Table 1. Mineral composition of five selected ash samples (van Heugten et al., 2006)

Feedstocks Processed		**Mineral Composition, dry matter basis**						
		%					**ppm**	
Type	**Source**	**Ash**	**P**	**K**	**Ca**	**Mg**	**Zn**	**Cu**
Swine manure	BGP Gasifier	88.8	12.72	10.06	14.24	4.41	8027	550
Swine manure	BKT Gasifier	60.7	9.38	7.36	10.03	3.32	2952	601

Table 2. Apparent digestibility of energy and P in growing pigs fed different sources of ash (van Heugten et al., 2006).

	Diet type		
Variable	**BKT**	**BGP**	**SEM**
P source digestibility, %[d]			
P	56.8[a]	76.5[a]	11.4

This study highlighted that the combustion method used significantly influenced the phosphorus digestibility in ashed swine manure solds, emphasizing the importance of optimizing processing techniques to maximize its nutritional value. These results also suggest that the completeness of combustion may be linked to ash digestibility, with more pure ash exhibiting better digestibility. Such studies underscore the potential of repurposing swine waste as a viable nutrient source in animal agriculture, aligning with circular economy principles by reducing waste and enhancing resource efficiency. By integrating sludge-derived phosphorus into feed formulations, producers could reduce costs associated with mineral supplementation while addressing environmental concerns associated with sludge disposal. Further research and technological advancements will be essential to refine these methods and ensure their feasibility for widespread adoption in the swine industry.

CHAPTER II: Study 1
Use of recycled ash from swine waste as a potential alternative dietary phosphorus source for pigs

Introduction

Swine lagoon sludge, formed as a result of the gradual accumulation of inorganic materials during standard lagoon operations, presents a challenge to lagoon treatment efficacy when solids accumulate excessively (Szogi et al., 2018). Regular removal of sludge is imperative for aging lagoons and those slated for closure, as it helps maintain treatment efficiency and cleanup in case of closure. The sludge itself is rich in various minerals, including calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), sulfur (S), sodium (Na), zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu)(Owusu-Twum & Sharara, 2020b). Of particular significance is P, the third most costly nutrient in swine diets, holding substantial financial value. The recycling of animal waste, including lagoon sludge, after drying or ashing may be a sustainable practice, reclaiming these mineral resources and reintegrating them into the production cycle, mitigating environmental concerns. As reviewed by Owusu-Twum & Sharara (2020b), the analysis of swine lagoon sludge samples from 27 lagoons showed an average of 3.83 g/L of P and 9.02% total solids (25 lagoons) representing approximately 4 to 5% of P on a dry matter basis. While these levels can vary based on sampling factors, the P content within swine lagoon sludge presents a possible renewable resource, fostering a circular economy through the efficient recovery and recycling of nutrients. While the current utilization of animal and poultry waste is primarily as fertilizer, incorporating them into swine diets as a mineral supplement could offer a greater value. Ashing swine lagoon sludge serves a dual purpose. First, minerals are concentrated by approximately two-fold, reducing the dietary inclusion rate and reducing transportation costs. Second, ashing lagoon sludge ensures the elimination of bacteria and viruses, rendering the resulting product sterile (Hoenig, 2005).

The objective of this study was to determine the chemical composition of ash derived from swine lagoon sludge after thermal treatment and the evaluation of the apparent total tract digestibility and bioavailability of P in the ashed swine lagoon sludge compared to monosodium phosphate (MSP) as the standard.

Materials and methods

Animal housing and experimental design

This study was conducted at the Swine Nutrition Facility at North Carolina State University (Raleigh, NC) in accordance with the protocol approved by the NCSU Institutional Animal Care and Use Committee.

A total of 56 pigs (Smithfield Premium Genetics) with an initial body weight of 44.14 ± 4.11 kg were individually assigned within sex and body weight blocks to pens measuring 1.80 x 0.94 m. Pens were randomly assigned to 7 dietary experimental treatments within each block, resulting in 8 blocks per treatment. Dietary treatments consisted of a negative control (NC) diet formulated to contain 40% of the suggested P requirements (NRC, 2012) and the NC diet supplemented with either MSP or ashed swine lagoon sludge (ASLS) to provide an additional 0.05%, 0.10%, and 0.15% of total P while maintaining a constant calcium-to-phosphorus (Ca:P) ratio of 1.1:1. MSP served as the standard P source with an assumed P availability value of 100% (NRC 2012; Traylor et al., 2005).

Ash preparation, diet formulation, sample collection

To prepare the ash from dried swine lagoon sludge, a combustion chamber was developed at the NC State Waste Management Center (Raleigh, NC) using a containment vessel, a blower and a propane torch. Dried ground sludge was added to the combustion chamber in 9 kg batches. The dried-ground sludge was first heated for three minutes with a propane torch, and combustion was then enhanced by airflow introduced through a blower, reaching a peak temperature of 1800°C, resulting in complete combustion of the input material. This process yielded 45-55% of the original material as ash. This ash was subsequently ground through a hammer mill using imperial sizes of screens for 1 mm particle size of the final product. Representative samples of the resulting ASLS were analyzed to determine mineral content. The analyzed composition of ASLS was used to formulate the experimental dietary treatments.

Experimental diets were created from a common corn-soybean meal based basal mix to meet the nutritional requirements outlined by NRC (2012), except for P and Ca. The basal mix was divided into seven portions and MSP, ASLS, limestone, salt, and fine sand were added to achieve the final experimental diets, while keeping other ingredients at equal levels (Table 3). Limestone was used at appropriate levels to ensure a constant Ca:P ratio of 1.1:1. Salt was incorporated to keep consistent Na levels across all diets, compensating for the Na derived from MSP. Fine sand was utilized as a filler to maintain inclusion levels of all other ingredients constant among all diets. Titanium dioxide was included in all diets at a 0.3% inclusion rate as an indigestible marker for determining apparent and standardized total tract nutrient digestibility. The diets were formulated using the National Swine Nutrition Guide Formulation Software (NSNG, 2010) and manufactured at the North Carolina State University Feed Mill Education Unit (Raleigh, NC) in meal form. Representative samples of the diets were collected and analyzed in duplicate at the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratory for chemical verification.

Table 3. Composition of the experimental diets for Experiment 1 (as-fed basis).

		Added P from MSP, %			Added P from SLS ash, %		
Ingredient	**0.00**	**0.05**	**0.10**	**0.15**	**0.05**	**0.10**	**0.15**
Corn, 7.5% CP	74.42	74.42	74.42	74.42	74.42	74.42	74.42
Soybean meal, 47% CP	19.81	19.81	19.81	19.81	19.81	19.81	19.81
Poultry fat	1.50	1.50	1.50	1.50	1.50	1.50	1.50
L-lysine HCl	0.355	0.355	0.355	0.355	0.355	0.355	0.355
DL-methionine	0.103	0.103	0.103	0.103	0.103	0.103	0.103
L-threonine	0.122	0.122	0.122	0.122	0.122	0.122	0.122
Titanium dioxide	0.300	0.300	0.300	0.300	0.300	0.300	0.300
Trace-mineral remix[1]	0.070	0.070	0.070	0.070	0.070	0.070	0.070
Vitamin premix[2]	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Sand[3]	1.686	1.448	1.210	0.973	1.123	0.562	0.000
Limestone[4]	0.947	1.079	1.211	1.343	0.961	0.975	0.989
Salt	0.397	0.302	0.208	0.113	0.396	0.394	0.393
Monosodium phosphate[3]	0.239	0.439	0.640	0.840	0.239	0.239	0.239
Ashed SLS[4]	0.000	0.000	0.000	0.000	0.549	1.098	1.648
Calculated composition							
ME, Mcal/kg	3.36	3.36	3.36	3.36	3.36	3.36	3.36
Crude protein, %	15.36	15.36	15.36	15.36	15.36	15.36	15.36
Total lysine, %	1.05	1.05	1.05	1.05	1.05	1.05	1.05
Calcium, %	0.451	0.501	0.551	0.601	0.501	0.551	0.601
Total phosphorus, %	0.404	0.454	0.504	0.554	0.454	0.504	0.554

Table 3. (Continued)

Available P	0.12	0.17	0.22	0.27	0.17	0.22	0.27
SID Amino acids							
Lys %	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Thr %	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Met %	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Met+Cys%	0.55	0.55	0.55	0.55	0.55	0.55	0.55
Trp %	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Ile %	0.53	0.53	0.53	0.53	0.53	0.53	0.53
Val %	0.61	0.61	0.61	0.61	0.61	0.61	0.61
Sodium %	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Chlorine %	0.28	0.23	0.17	0.11	0.28	0.28	0.28
Magnesium %	0.16	0.17	0.17	0.17	0.19	0.21	0.23
Potassium %	0.67	0.67	0.67	0.67	0.67	0.67	0.68

[1]Supplied per kg of basal diet: 118 mg of Ca as $CaCO_3$, 51 mg of Zn as $ZnSO_4$, 51 mg of Fe as $FeSO_4$, 15 mg of Mn as $MnSO_4$, 8 mg of Cu as $CuSO_4$, 0.14 mg of I as Ca $(IO_3)_2$ and 0.14 mg of Se as Na_2SeO_3.
[2]Supplied per kg of basal diet: 10,417 IU of vitamin A as retinyl acetate, 1,458 IU of vitamin D_3 as cholecalciferol, 104 IU of vitamin E as dl-α-tocopheryl acetate, 37.5 mg of niacin as nicotinic acid, 31 mg of pantothenic acid as calcium D-pantothenate, 8 mg of riboflavin as riboflavin, 5 mg of pyridoxine as pyridoxine hydrochloride, 4 mg of vitamin K as 2-methyl-1,4 naphthoquinone-2-sodium bisulfite, 4 mg of folacin as folic acid, 2.5 mg of thiamine as thiamine mononitrate, 0.5 mg of biotin as D-biotin, 0.03 mg of vitamin B_{12} as cyanocobalamin, and 0.17 mg chromium as chromium propionate.
[3]XP-4 Monosodium Phosphate, Eastern Minerals, Henderson, NC.
[4]Ground dried sludge was ashed through combustion (Table 4 details mineral content).

Throughout the study, pigs were limit-fed at 3 times maintenance requirements, calculated as 197 kcal ME/kg $BW^{0.60}$ (NRC, 2012) within each weight block. Pigs were fed twice daily at 8:00 and 16:00 with unlimited access to water provided. Pigs were individually weighed on days 0, 14, 21, 28 and 35 to determine body weight and daily gain. They were observed daily to ensure they remained healthy, active, and consumed all feed provided with free access to water. During the final three days of the study, fresh fecal samples were collected daily from each pig, pooled within pig, and stored at -20°C for subsequent processing and analysis. The pooled samples were dried at 64°C for a minimum of 72 hours in a conventional drying oven. The dried samples were placed in plastic zip-lock bags and stored in a climate-controlled environment until they were ground to a consistent particle size for further analysis.

On the final day of the study (day 35), pigs were euthanized using a captive bolt gun followed by exsanguination. Blood samples were obtained at this time for serum separation through centrifugation at 900 x g for 20 minutes at 20°C. The serum samples were then stored at -80°C for subsequent analysis. The right front foot of each pig was collected and stored at -20°C. The 3rd metacarpal bones were dissected for analysis of bone composition and bone strength. The 3rd metacarpal bones were carefully cleaned using a scalpel, and their length and width at the bone's center were measured with a digital caliper. Bone strength was assessed using a texture analyzer (Model: TA.XT Plus, Texture Analyzer Technologies, Scarsdale, NY, USA) through a three-point bending test, featuring 5 cm between supports and a 250 kg load cell. Peak breaking strength was recorded, signifying the maximum load supported before failure. Post-breaking, the bones were weighed and defatted by a 72-hour soak in petroleum ether. Following this, they were transferred into a crucible, weighed again, and then ashed in a muffle furnace at 600˚C for 10 hours. Ash weights were recorded and ash calculated as a percentage of the defatted bone weight.

Chemical analyses

Samples of ashed swine lagoon sludge (ASLS) were submitted to Midwest Laboratories Inc. (Omaha, NE) and analyzed in triplicate to determine concentrations of P, Ca, S, K, Mg, Na, Fe, Mn, Cu, and Zn (Table 4).

Table 4. Analyzed concentrations in triplicate of minerals in ashed swine lagoon sludge for Experiment 1 (as is basis)

Minerals	**MEAN[1]**
Phosphorus, %	9.16
Calcium, %	8.30
Magnesium, %	4.28
Potassium, %	0.51
Sodium, %	0.19
Sulfur, %	0.18
Iron, ppm	14833
Zinc, ppm	5523
Copper, ppm	2717
Manganese, ppm	1427

[1]Each value represents the mean of the analyzed concentration of minerals in triplicate for ash

Feed samples (Table 5) and fecal samples were submitted to the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO) for proximate analyses, including crude protein (AOAC Official Method 990.03, 2006), moisture, crude fiber (AOAC Official Method 978.10, 2006), crude fat (AOAC Official Method 920.39 (A)), ash (AOAC Official Method 942.05), P (AOAC Official Method 966.01, 2006) and titanium dioxide (Myers et al., 2004).

Table 5. Analyzed nutrient content of experimental diets (%, DM basis)[1]

Item	Basal NC	Added P from MSP[2] 0.5%	1.0%	1.5%	Added P from ASLS[2] 0.5%	1.0%	1.5%
Crude fiber	2.00	1.95	1.94	1.87	1.94	1.91	2.27
Crude protein	18.03	16.98	17.20	17.93	17.42	17.70	17.70
Crude fat	3.88	4.24	4.47	4.79	4.75	4.68	4.55
Ash	7.00	6.64	6.25	6.32	6.15	6.04	6.18
Titanium dioxide	0.12	0.14	0.13	0.15	0.15	0.14	0.14
Phosphorus	0.43	0.44	0.50	0.56	0.45	0.51	0.60

[1]Each value represents the mean of duplicate analysis.
[2]Diets with 0.05%, 0.10%, or 0.15% P added to the Negative control (NC) from monosodium phosphate or diets with 0.05%, 0.10%, or 0.15% P added to the NC from ashed swine lagoon sludge.

Serum samples were submitted to Antech Diagnostics (Cary, NC) for serum chemistry analysis (total protein, albumin, globulin, albumin/globulin ratio, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, 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.

Calculations and statistical analysis

The statistical analysis for this study was conducted using JMP Pro 17 software (SAS Institute, Inc). The experimental units were the individual pigs, and they were blocked in a randomized complete block design. The randomization of blocks was based on the sex and initial body weight of each pig, ensuring an equally balanced and controlled distribution across treatments. Diet analysis showed that the negative control (NC) diet exceeded targeted Ca and P concentrations, therefore, NC was excluded from the final statistical analysis. Growth performance data were analyzed using a 2 x 3 factorial arrangement, with P source (MSP or ASLS) and P supplementation level (0.05, 0.10, 0.15%) as main effects, along with their interactions and block effects included in the model. Serum chemistry, including P concentrations, P excretion, apparent and standardized total tract digestibility of P, third metacarpal breaking force, bone dry matter, and bone ash were analyzed using a multiple linear regression model accounting for the effects of block and P supplementation. The estimated bioavailability of P in ASLS compared to the standard MSP was calculated using a slope ratio assay. Statistical significance differences were considered at $P \leq 0.05$ with tendencies at $0.05 < P \leq 0.10$.

Results and discussion

Growth performance

Statistical analysis indicated that final body weight (BW) and overall average daily gain (ADG) were not influenced by the main effect of dietary P level (P=0.27), the main effect of P source (P=0.87), or their interaction effect (P=0.63) (Table 6). The effects of dietary P from ASLS on body weight align with the study conducted by McAuley (2022), where overall body weight was not influenced by dietary P supplementation from dried sludge, but body weight linearly increased with the addition of P from MSP. In this study, the diets were formulated to be below P requirements by NRC (2012), and even at the highest supplemental level, P intake remained under NRC guidelines, which would lead to an expected response in body weight due to the deficiency. Results on body weight from this study suggest that increasing dietary P from either MSP or ASLS did not impact body weight in growing pigs (Figure 4). These results are similar to those reported and reviewed by Ryan et al. (2011), where no differences were observed in growing pigs fed different P levels below the recommended requirements over a 35-day period, and similar effects have been reported by others (Hastad et al., 2004) where no significant impact was observed in growing pigs over a 28-days period. Phosphorus from different sources may differ in digestibility and absorption by pigs, leading to variations in its bioavailability (Zhai et al., 2022). The literature reviewed by Misiura et al. (2020) indicates that providing pigs with diets containing P below the recommended requirements may not compromise average daily gain but can negatively impact P deposition in bones

Table 6. Response of growth performance (kg) to supplemental dietary P from MSP and ASLS sources

	MSP[1]			**ASLS**[1]				*P-values*[2]		
	0.05	**0.10**	**0.15**	**0.05**	**0.10**	**0.15**	SE	Level	Source	Level *Source
IBW[3]	44.02	44.00	44.57	44.10	43.77	44.08	*	*	*	*
FBW[4]	73.91	74.96	74.19	73.86	74.30	73.80	1.66	0.27	0.87	0. 63
ADG[5]	0.852	0.882	0.839	0.851	0.863	0.853	0.05	0.27	0.87	0. 63

[1] Each value represents the least squares mean of eight pigs per treatment over a 35 day period.
[2] P-value represents main and interaction effects of P sources and increased dietary phosphorus via monosodium phosphate (MSP) or ashed swine lagoon sludge (ASLS) when added to the negative control diet (NC).
[3]Initial body weight (IBW), [4] Final body weight (FBW), [5]Average daily gain (ADG)

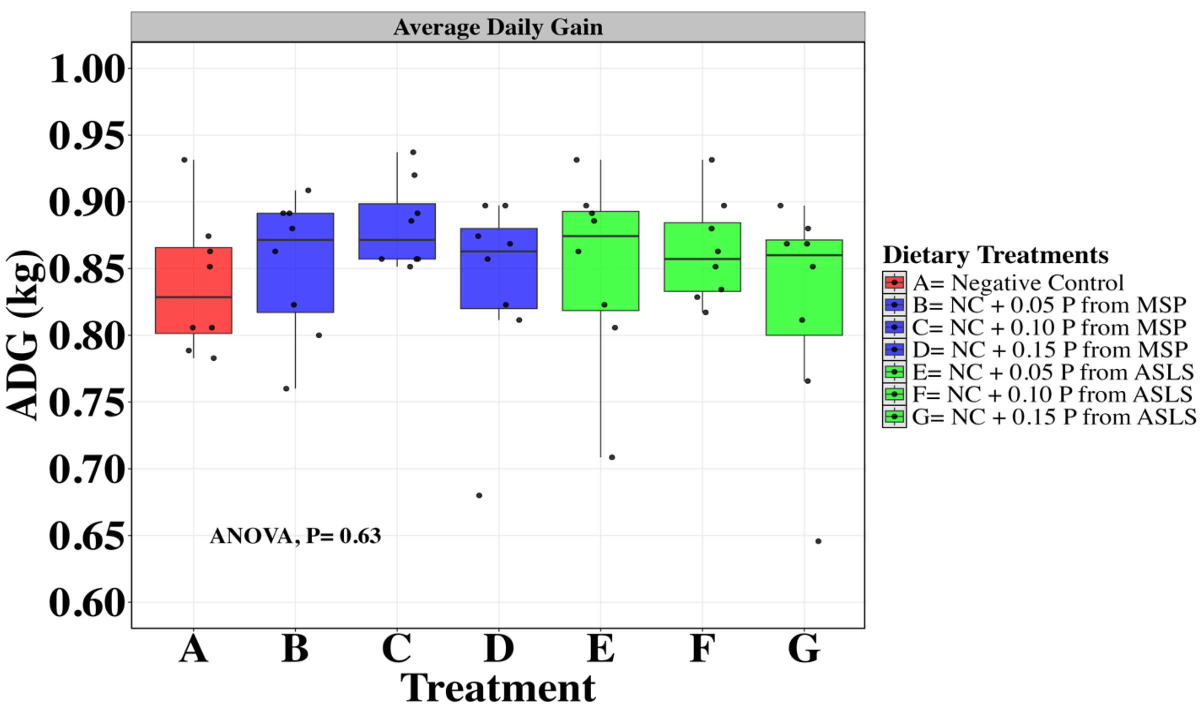


Figure 4. Average daily gain of pigs fed supplemental P from either MSP or ASLS

Serum chemistry

Serum P concentrations linearly increased (P = 0.022) in pigs supplemented with increasing levels of P from MSP (8.13, 8.53, and 8.90 mg/dL) (Figure 5). A similar trend was observed with ASLS supplementation, where serum P concentrations tended to increase (P = 0.098; 8.26, 8.64, and 8.79 mg/dL) with increasing P from ASLS (Table 7). These results are comparable with the study by McAuley (2022) where serum P concentrations increased with the incremental addition of dietary P from MSP and tended to increase in dried swine lagoon sludge as P source. Vötterl et al. (2021) suggested that higher available P intake increased serum P concentrations, suggesting that serum P is a sensitive marker of P availability. However, other factors also influence serum P levels, including the regulation of P reabsorption by the kidneys (Amanzadeh & Reilly, 2006). The kidneys may maintain serum P balance, regardless of P intake (Raikou, 2021). Serum P concentrations measured in the present study indicate that P in ASLS was less bioavailable compared to MSP. Serum K concentrations increased linearly in pigs fed MSP diets (P = 0.01; 5.19, 5.23, and 5.83 mEq/L), whereas no significant changes were detected in pigs receiving ASLS diets (4.98, 5.09, and 5.01 mEq/L). Furthermore, serum Na (145.4, 145.6, and 147.3 mEq/L) and Cl concentrations (101.1, 101.8, and 103.9 mEq/L) increased linearly in pigs fed MSP diets, but not in pigs supplemented with ASLS. The observed increase in chloride levels in MSP supplementation can be attributed to the adjustments made to maintain consistent sodium levels across diets. Since MSP contributes sodium, reducing its inclusion required compensatory sodium supplementation using NaCl (salt). As a result, lower MSP diets contained more NaCl, which introduced additional chloride. This unintended increase in chloride likely influenced the dietary electrolyte balance. The linear increase in serum sodium with MSP supplementation, despite sodium adjustments, may be explained by shifts in electrolyte homeostasis due to varying Cl^-

levels. Higher dietary Cl can alter renal sodium handling, potentially increasing sodium retention or modifying excretion patterns to maintain acid-base balance (DiBartola, 2011). Additionally, changes in the strong ion difference could have affected osmotic regulation and fluid balance, indirectly influencing serum sodium levels. Sodium, as the primary extracellular cation, is crucial for regulating osmotic pressure, extracellular fluid volume, and plasma volume. Similarly, chloride, often paired with sodium, is the main extracellular anion contributing to osmotic balance, fluid regulation, essential for maintaining acid-base balance, and serves as a key component of gastric hydrochloric acid, facilitating digestion (Institute of Medicine, 2005). This suggests that MSP functions not only as a phosphorus source but also plays a significant role in shaping the dietary electrolyte balance, with potential effects on nutrient absorption and systemic homeostasis

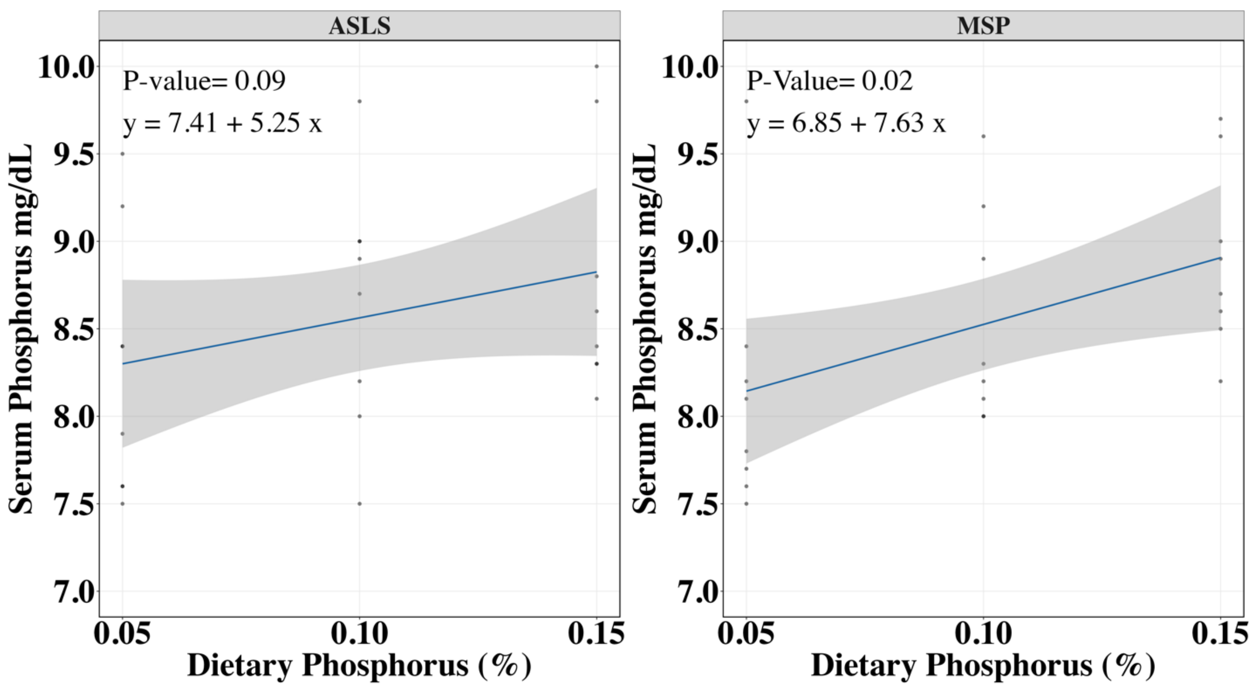


Figure 5. Regression plot of serum P concentrations in response to the addition of dietary P from either ASLS or MSP.

Table 7. Response of serum chemistry[3] to supplemental dietary P from MSP and ASLS sources on day 35 on test[1]

	MSP					ASLS				
Source	**0.05**	**0.10**	**0.15**	*SE*	*P-value*[2]	**0.05**	**0.10**	**0.15**	*SE*	*P-value*[2]
T.Protein (g/dL)	5.63	5.50	5.56	0.39	0.75	5.55	5.38	5.53	0.28	0.93
Albumin (g/dL)	3.55	3.48	3.53	0.33	0.88	3.54	3.54	3.46	0.32	0.64
Globulin (g/dL)	2.08	2.01	2.03	0.28	0.80	1.90	1.85	2.08	0.22	0.13
A/G ratio	1.74	1.78	1.75	0.34	0.94	1.84	1.94	1.76	0.38	0.70
P (mg/dL)	8.14	8.54	8.9	0.43	0.02	8.26	8.64	8.79	0.48	0.09
Ca (mg/dL)	10.0	9.62	9.83	0.44	0.47	9.61	9.39	9.79	0.51	0.51
Mg(mEq/L)	1.55	1.71	1.61	0.11	0.28	1.64	1.54	1.60	0.18	0.68
K(mEq/L)	5.19	5.23	5.83	0.45	0.01	4.98	5.09	5.01	0.55	0.89
Na(mEq/L)	145.4	145.6	147.3	1.60	0.03	145.8	146.8	144.1	2.03	0.13
Na/K ratio	28.55	27.88	24.83	1.33	<.01	29.50	29.0	29.25	3.06	0.87
Cl (mEq/L)	101.1	101.8	103.9	1.56	<.01	101.1	101.4	101.3	1.03	0.71
BUN (mg/dL)	5.50	5.63	6.0	0.57	0.82	6.0	6.0	5.88	0.73	0.73
AST (IU/L)	32.0	32.36	31.38	10.3	0.90	34.25	38.13	33.50	10.8	0.89
ALT (IU/L)	34.5	29.75	30.75	4.39	0.11	30.63	35.75	32.25	7.71	0.68
GGT (IU/L)	40.13	42.42	41.42	3.60	0.43	38.62	44.09	38.25	6.53	0.91
SDMA (UG/dL)	13.14	16.01	14.79	2.22	0.16	15.36	15.05	12.69	1.98	0.02
Glucose (mg/dL)	82.12	82.62	83.62	8.43	0.73	79.50	76.00	79.00	5.21	0.85
Creatine (mg/dL)	1.26	1.27	1.26	0.12	0.53	1.24	1.24	1.20	0.09	1.00
AP. (IU/L)	172.5	168.4	160.4	17.2	0.70	178.6	183.9	163.8	31.9	0.37

[1]Each value represents the least squares mean of eight pigs per treatment group over a 35-day period.

[2]P-value represents linear effects of increased phosphorus via monosodium phosphate (MSP) or ashed swine lagoon sludge (ASLS) when added to the negative control diet (NC).

[3]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 phosphatase48 (AP), gamma-glutamyl transferase (GGT), and symmetric dimethylarginine (SDMA).

Phosphorus digestibility and excretion

Fecal concentrations (DM basis) of P did not increase linearly with the addition of P from MSP (P = 0.95; 1.95, 2.13, and 2.24%) but they did increase with added dietary P from ASLS (P = 0.003, 2.05, 2.08, and 2.32%). However, there were no significant differences (P = 0.809) between sources (Figure 6). Apparent total tract digestibility (ATTD) of P was calculated using titanium dioxide as an indigestible marker in accordance with Zhai & Adeola (2012). The apparent total tract digestibility of P did not increase linearly (P= 0.97) with the incremental addition of P from MSP (39.30, 48.23, and 48.56%) but it did increase with ASLS (40.67, 46.39, and 48.17%) as the main P source (Figure 7). No significant differences were detected between sources at their corresponding inclusion levels (P=0.722; Table 8).

Since ATTD does not account for endogenous P losses (EPL), standardized total tract digestibility (STTD) was also calculated to provide a more accurate estimate of P digestibility. Although EPL was not directly measured, a reference value of 190 mg/kg dry matter intake (DMI) from National Research Council (NRC) (2012) was used for standardization. The STTD of P (Table 8) increased linearly (P<0.001) with dietary P addition from ASLS but not for MSP and there were no differences between P sources. The lack of a linear increase in ATTD, and STTD of P with incremental P from MSP suggests a potential saturation of absorption causing a plateau effect (Jongbloed, 1987), which could occur for P in MSP which is highly bioavailable and intestinal P transport reaches its capacity. However, this response was unexpected, given that dietary P levels were formulated below requirements, where an increase in digestibility would typically be anticipated. The higher STTD values compared to ATTD account for endogenous losses, which is particularly important in this study due to the use of P-deficient diets where endogenous losses contribute a larger proportion of total P excretion compared to scenarios where dietary P exceeds requirements. In contrast, when dietary P is in excess, fecal P excretion primarily reflects unabsorbed dietary P, reducing the relative impact of endogenous losses on total excretion (Valk et al., 2002). These results support that the STTD of P in ASLS is similar to that in MSP, which aligned with findings indicated in a previous study by van Heugten et al. (2006) which highlighted the potential of ash-derived P as a viable dietary P source for swine. Furthermore, as reported by van Heugten et al. (2006), the process of combustion used to produce ASLS may significantly influence P digestibility. In that study, different gasifiers with varying designs were used to ash various feedstocks and showed that the combustion altered the chemical composition of the ash, with those that had more complete combustion of ash typically exhibiting better digestibility. The inclusion of other minerals should be considered when adding ash-derived ingredients to diets, as they may influence P digestibility. Microminerals such as magnesium or iron may form complexes which competes and affects the absorption, which might result in reduced P digestibility (Richard Baker & Stein, 2010). Sodium plays a crucial role in the active transport of P across the intestinal membrane, and inadequate Na levels may impair P absorption (Sabbagh et al., 2011). Given the mineral-rich composition of ASLS, these interactions should be evaluated in the future to better understand their potential impact on P digestibility.

Table 8. Response of apparent total tract digestibility (ATTD)[1] and excretion of P (dry matter basis)[1] to supplemental dietary P from MSP and ASLS sources

	MSP					ASLS				
Source	**0.05**	**0.10**	**0.15**	*SE*	[2]*P-value*	**0.05**	**0.10**	**0.15**	*SE*	[2]*P-value*
ATTD of P	39.30	48.23	48.56	4.27	0.97	40.67	46.39	48.17	2.75	<0.01
STTD of P	45.10	52.10	51.90	4.28	0.99	44.91	50.11	51.31	2.79	<0.01
P excreted	1.95	2.13	2.24	0.26	0.95	2.05	2.08	2.32	0.15	0.003

[1]Each value represents the least squares mean of eight pigs per treatment group over a 35-day period
[2] P-value represents linear effects of increased Phosphorus via monosodium phosphate (MSP) or swine lagoon sludge (ASLS) when added to the negative control diet (NC).

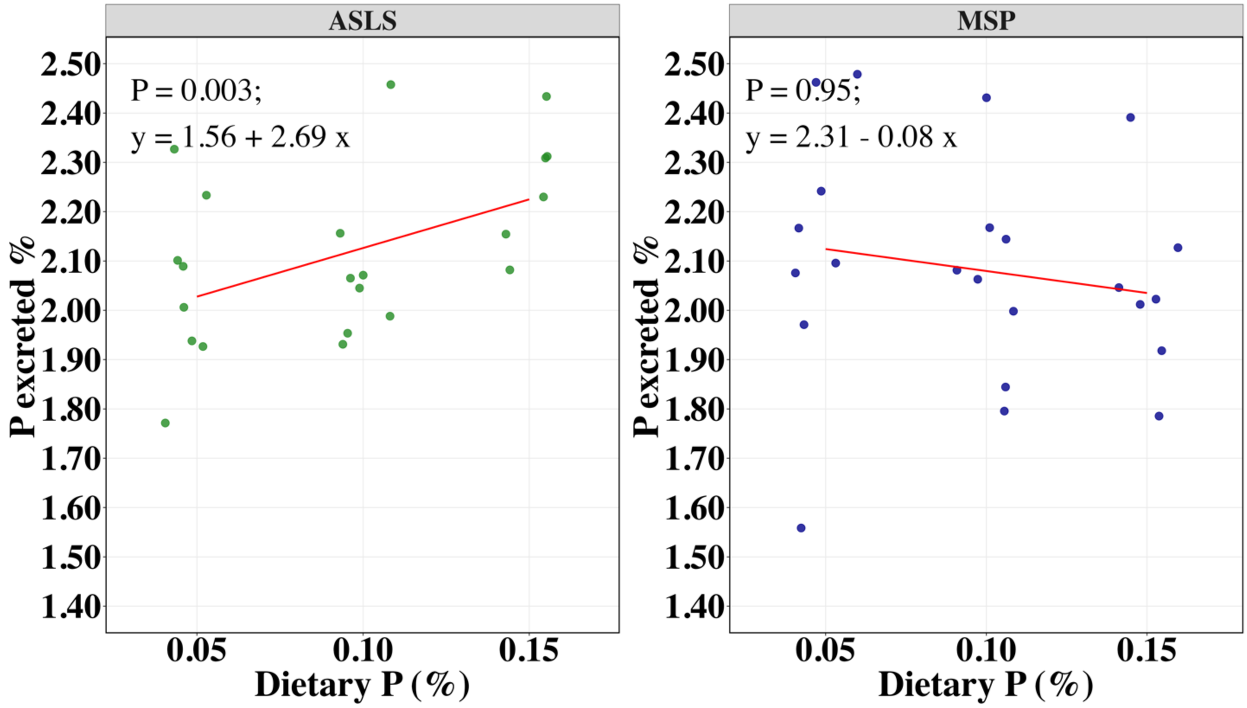


Figure 6. Regression plot of analyzed P excretion in response to the addition of dietary P from ASLS and MSP.

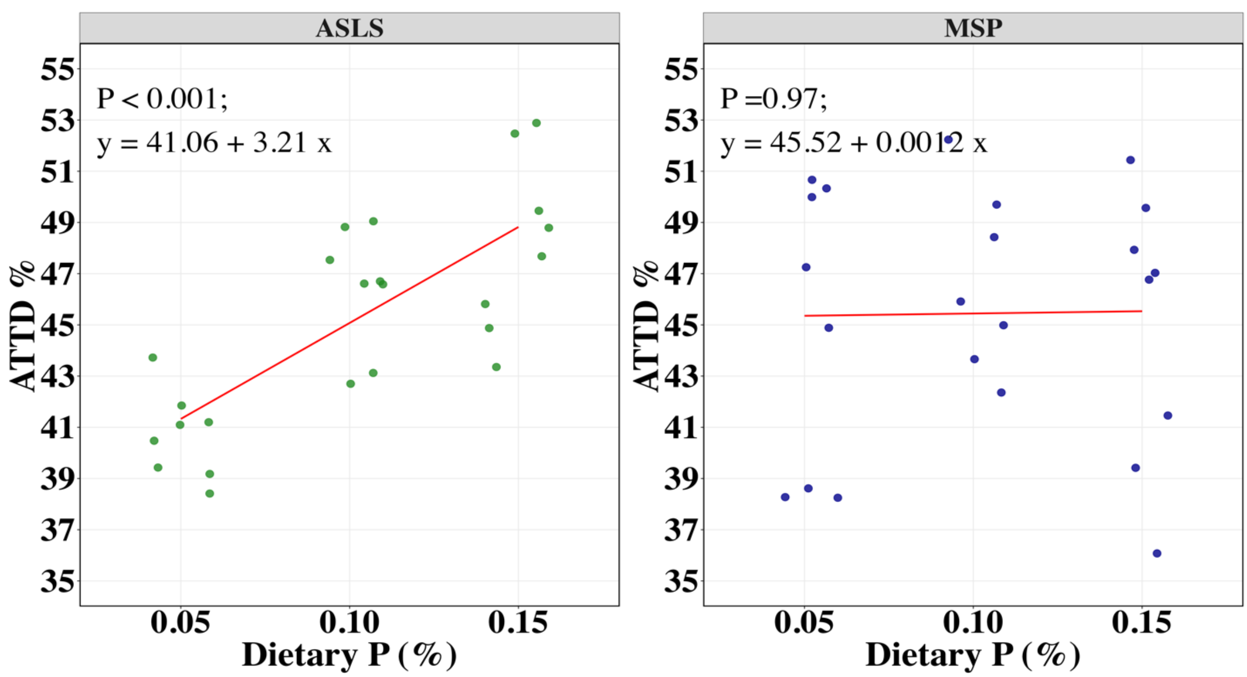


Figure 7. Regression plot of apparent total tract digestibility (ATTD) of P in response to the addition of dietary P from ASLS and MSP.

Third metacarpal bone analysis

Peak breaking force refers to the maximum load or force that a bone can withstand before it fractures or breaks under stress (Crenshaw et al., 1981). The third metacarpal bone-breaking strength (Table 9) linearly increased with the addition of P from MSP ($P<0.001$), but was not impacted with increasing dietary P from ASLS ($P=0.695$) suggesting that P from ASLS had a lower bioavailability compared to MSP and pigs were unable to efficiently absorb and utilize it for bone formation. Numerous studies have demonstrated that increasing dietary P intake leads to higher levels of hydroxyapatite in bone (Penido & Alon, 2012), a key component responsible for providing bone mineral density resulting in causing an increase in peak-breaking force, likely due to improved P utilization for bone strengthening.

Dry matter of the third metacarpal bone was not affected by the addition of P from MSP ($P= 0.695$; 67.46, 67.18, and 68.45 %) and or ASLS ($P =0.23$; 67.19, 67.68, and 67.95 %). However, bone ash linearly increased ($P=0.012$) with additional P from ASLS (47.07, 48.20, and 48.70%), but not from MSP ($P=0.135$; 47.57, 48.03, and 48.52%) (Table 9). The increase in bone ash content might indicate an increase in mineral deposition and bone mineralization (Hakami et al., 2022). However, this may not have been accompanied by improved incorporation into the bone matrix to enhance mechanical strength. This could be attributed to a lower absorption and utilization of P from ASLS by the pigs.

Table 9. Response of bone composition[1] to supplemental dietary P from MSP and ASLS sources

	MSP					ASLS				
Source	**0.05**	**0.10**	**0.15**	*SE*	[2]*P-value*	**0.05**	**0.10**	**0.15**	*SE*	[2]*P-value*
Peak force	75.06	99.90	114.9	20.59	<.001	98.14	96.86	102.1	16.28	0.70
Dry matter	67.19	67.68	67.95	1.21	0.23	67.46	67.18	68.45	1.50	0.24
Bone ash	47.57	48.03	48.52	1.25	0.15	47.07	48.20	48.70	1.08	0.008

[1]Each value represents the least squares mean of eight pigs per treatment group over a 35-day period
[2] P-value represents linear effects of increased phosphorus via monosodium phosphate (MSP) or ashed swine lagoon sludge (ASLS).

Slope ratio and relative bioavailability

Analyzed P concentrations in serum were used as criteria to estimate P bioavailability in ASLS compared to MSP. The bioavailability was estimated using the ratio of the slopes derived from the regression equations of serum P levels against dietary P for each source. For ASLS, the regression equation was [serum P = 7.40 + 5.25 × dietary P, R^2 = 0.09], while for MSP the equation was [serum P = 6.84 + 7.62 × dietary P, R^2 = 0.22]. The slope ratio between ASLS and MSP suggested a relative bioavailability of 68.89% for P in ASLS compared to MSP. Both the intercepts and slopes of these regressions were found to be statistically significant (Table 10). However, a statistical test comparing the slopes between ASLS and MSP revealed no significant difference (P = 0.58). This suggests that accounting for the variation, the bioavailability of P in ASLS is equivalent to that of MSP, indicating that ASLS provides P with similar digestibility and absorption efficiency as the established phosphorus source. Serum P levels can serve as an important criterion for nutritionists to calculate the bioavailability of P in alternative mineral sources compared to established standards (Peñuela Sierra et al., 2013). However, serum P may not fully reflect the bioavailability of P for bone mineralization. Bone ash content and peak breaking force are commonly used for assessing the bioavailability of P because it directly measures the impact of P on bone mineralization and mechanical strength (Petersen et al., 2011). However, they were not used as criteria for estimating bioavailability in this study because the regression analysis for one of the phosphorus sources did not show a significant linear slope. Consequently, the slope ratio method used to estimate relative bioavailability would be invalid, as a non-significant slope prevents a meaningful comparison between the sources (Ammerman, 1995).

Table 10. Regression parameters for phosphorus bioavailability between ASLS and MSP with serum P as the criterion.

	MSP				**ASLS**			
Parameter	**Est.**	**SE**	***P-value***	**C.I 95%**	**Est.**	**SE**	***P-value***	**C.I 95%**
Intercept	6.85	0.61	<0.01	(5.55, 8.14)	7.41	0.53	<0.01	(6.27, 8.54)
Slope	7.63	2.72	0.01	(1.83, 13.42)	5.25	2.38	0.04	(0.17, 10.33)

Estimates (Est.), 95% Confidence Interval (C.I 95%)
P-value for intercept tests whether the estimated intercept differs significantly from zero.
P-value for slope tests whether the estimated slope differs significantly from 1

Conclusions

This study aimed to evaluate the chemical composition of ash derived from swine lagoon sludge produced through thermal processing and to assess the apparent digestibility and bioavailability of phosphorus in this material. Chemical analysis suggests a concentration of 9.16% of P in ashed swine lagoon sludge, which is the main nutrient of interest to provide potential economic benefits. Results of slope-ratio analysis using P in serum as criteria indicated that ASLS has a bioavailability of 68.89% compared to MSP as the standard and can be used as an alternative dietary P source if approved as a feed ingredient. Additionally, ATTD of P was similar when dietary P was supplemented from ASLS compared to MSP. However, measurements of bone composition did not fully support the value of dietary P from ASLS compared to MSP. Further research is needed to establish a more precise bioavailability value for ASLS, as serum phosphorus was the only criterion used in this study. Additionally, the exclusion of the negative control (NC) group from statistical analysis, due to exceeding the targeted dietary P levels reduced the accuracy of the slope-ratio estimation. Future studies should incorporate additional response criteria and refine experimental designs to improve the reliability of bioavailability assessments for ASLS as P source.

CHAPTER III: Study II
Practical evaluation of the value of feeding phosphorus recycled from swine waste in nursery pigs

Introduction

Phosphorus (P) is a critical mineral in swine diets, supporting a range of vital biological functions, especially in the development and maintenance of the skeletal system. It is also key to the formation of phospholipids, enzymes, and nucleic acids, all of which are essential for proper cellular function and metabolism (Berndt & Kumar, 2009). However, excessive phosphorus inclusion in diets can have significant environmental consequences. Elevated dietary P levels lead to increased phosphorus excretion, which contributes to the accumulation of nutrients in swine lagoons (Gonçalves et al., 2016). The management of these lagoons poses a growing challenge for swine producers, particularly small-scale farmers. While swine operations often recycle nutrients in sludge as fertilizer, this practice is not always feasible for those with limited land resources. Improperly managed swine lagoon sludge presents additional risks, including the potential for mineral runoff into water sources, which can lead to environmental contamination and associated health hazards, representing a major environmental concern (Philips, 2023). Emerging research highlights the potential for minerals in swine lagoon sludge to be bioavailable and digestible, creating opportunities for recycling these nutrients into swine diets making it a viable alternative to traditional mineral supplements. A recent study by McAuley (2022) reported that the bioavailability of P in dried swine lagoon sludge was 67.1% relative to monosodium phosphate (MSP) utilizing bone strength as the primary criterion for assessing P availability. Additionally, a previous study by van Heugten et al. (2006) reported that feeding ashed swine manure solids, produced through different combustion methods, effectively maintained growth performance and

P digestibility compared to dicalcium phosphate used as the standard. Phosphorus, being the third most expensive component in swine feed formulations and the second most abundant mineral in the body, represents a particularly valuable target for recovery. Therefore, the recycling of P from lagoon sludge in diets for swine might offer both environmental and financial benefits. However, practical evaluations are essential to confirm its efficacy in real-world feeding scenarios. Additionally, a comprehensive economic analysis is necessary to determine the feasibility and long-term sustainability of incorporating lagoon sludge-derived P into swine diets.

The objective of this study was to conduct a practical evaluation in nursery pigs to determine the effectiveness of ashed and dried swine lagoon sludge as an alternative P source compared to monocalcium phosphate. Dried sludge was included as it represents a common byproduct of conventional sludge management practices, while ashed sludge was assessed for its potential to provide a more concentrated nutrient source. Additionally, an economic assessment was conducted to assess the cost-effectiveness and potential benefits of incorporating these materials into swine diets

Materials and methods

Animal housing and experimental design

The experimental protocol for this study was approved by the NCSU Institutional Animal Care and Use Committee (IACUC protocol 21-467).

A total of 192 pigs (Smithfield Premium Genetics, 28 days old with an initial body weight of 6.99 ± 0.79 kg) were assigned to 48 pens with 4 pigs each balanced by gender. Pens were assigned to 16 blocks based on initial body weight, and the three experimental treatments were randomly allocated within each block.

P sources, diet formulation and sample collection

The dried sludge and combustion process from study 1 was replicated to produce the test phosphorus sources: dried swine lagoon sludge (DSLS) and ashed swine lagoon sludge (ASLS). Monocalcium phosphate served as the control phosphorus source. Representative samples of the resulting ashed swine lagoon sludge (ASLS), dried swine lagoon sludge (DSLS), and monocalcium phosphate were analyzed in quadruplicate to determine concentrations of phosphorus (P), calcium (Ca), sulfur (S), potassium (K), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn). These results and the bioavailability value of P in ASLS obtained from study 1 (68.79 %) and the value for DSLS (67.1%) reported by McAuley (2022) were utilized to formulate the experimental dietary treatments.

The dietary treatments included three experimental diets: (1) a negative control (NC) diet using MCP as the P source, (2) a diet with ASLS as the P source, and (3) a diet with DSLS as the P source. Diets were designed with available P at 80% of the requirements recommended by PIC (2016). The experimental feeding period was divided into two dietary phases consisting of diets fed from day 0 to 14 (Phase 1) and diets fed from day 14 to 28 Phase 2 (Table 10). Diets met or exceeded nutrient requirements estimates based on NRC (2012) with the exception of P.

Table 11. Composition of the experimental Phase 1 diets for Experiment 2 (as-fed basis)

Ingredients	MCP	ASLS	DSLS
Corn, 7.5% CP	55.12	53.54	50.64
Soybean meal, 47% CP	28.00	28.00	28.00
Whey, permeate	6.00	6.00	6.00
Processed soy protein[1]	4.00	4.00	4.00
Fish meal, menhaden	2.50	2.50	2.50
Poultry fat	1.00	1.00	1.00
L-lysine HCl	0.381	0.385	0.391
DL-methionine	0.199	0.204	0.212
L-threonine	0.248	0.252	0.260
L-tryptophan	0.044	0.045	0.046
L-valine	0.041	0.046	0.054
Limestone	0.957	0.751	0.617
Salt	0.351	0.351	0.351
Vitamin premix[2]	0.050	0.050	0.050
Trace mineral premix[3]	0.150	0.150	0.150
Monocalcium phosphate	0.957	0.000	0.000
Swine lagoon sludge, ashed[4]	0.000	2.723	0.000
Swine lagoon sludge, dried[5]	0.000	0.000	5.731
Calculated composition			
ME, Mcal/kg	3.39	3.33	3.23
NE, Mcal/kg	2.45	2.41	2.33
Crude protein, %	22.05	21.94	21.75
Calcium, %	0.80	0.80	0.80
Total phosphorus, %	0.69	0.74	0.74
Available phosphorus, %	0.36	0.36	0.36
SID Amino acids			
Lys %	1.40	1.40	1.40
Thr %	0.91	0.91	0.91
Met %	0.51	0.52	0.52
Trp %	0.28	0.28	0.28
Ile %	0.81	0.81	0.80
Val %	0.94	0.94	0.94

[1]HP300, Hamlet protein Inc, Findlay, Ohio.
[2]Supplied per kg of basal diet: 10,417 IU of vitamin A as retinyl acetate, 1,458 IU of vitamin D_3 as cholecalciferol, 104 IU of vitamin E as dl-α-tocopheryl acetate, 37.5 mg of niacin as nicotinic acid, 31 mg of pantothenic acid as calcium D-pantothenate, 8 mg of riboflavin as riboflavin, 5 mg of pyridoxine as pyridoxine hydrochloride, 4 mg of vitamin K as 2-methyl-1,4 naphthoquinone-2-sodium bisulfite, 4 mg of folacin as folic acid, 2.5 mg of thiamine as thiamine mononitrate, 0.5 mg of biotin as D-biotin, 0.03 mg of vitamin B_{12} as cyanocobalamin, and 0.17 mg chromium as chromium propionate
[3]Supplied per kg of basal diet: 118 mg of Ca as $CaCO_3$, 51 mg of Zn as $ZnSO_4$, 51 mg of Fe as $FeSO_4$, 15 mg of Mn as $MnSO_4$, 8 mg of Cu as $CuSO_4$, 0.14 mg of I as $Ca(IO_3)_2$ and 0.14 mg of Se as Na_2SeO_3.
[4]Ground dried sludge was ashed through combustion (Table 12 details mineral content).
[5]Sludge was greenhouse-dried and ground (Table 13 details mineral content).

Phase 2 diets consisted of a corn-soybean meal basal mix and titanium dioxide was included in all diets at a 0.3% inclusion rate as an indigestible marker for determining P digestibility (Table 11). All nutrients across diets were kept at equal levels, while corn was the primary ingredient that was adjusted to account for the different inclusion levels of P sources (MCP, ASLS, and DSLS).

Table 12. Composition of the experimental diets for Experiment 2 in Phase 2 (as-fed basis)

Ingredients	**MCP**	**ASLS**	**DSLS**
Corn, yellow dent	63.06	61.35	58.20
Soybean meal, 46.5% CP	32.00	32.00	32.00
L-valine	0.04	0.05	0.06
Poultry fat	1.00	1.00	1.00
L-lysine HCl	0.43	0.43	0.44
DL-methionine	0.17	0.18	0.19
L-threonine	0.20	0.21	0.22
L-tryptophan	0.04	0.04	0.04
Limestone	1.13	0.90	0.75
Salt	0.40	0.40	0.40
Vitamin premix[1]	0.05	0.05	0.05
Trace mineral premix[2]	0.15	0.15	0.15
Titanium dioxide	0.30	0.30	0.30
Monocalcium phosphate 21%	1.03	0.00	0.00
Swine lagoon sludge, ashed[3]	0.00	2.94	0.00
Swine lagoon sludge, dried[4]	0.00	0.00	6.20
Calculated composition			
ME - Mcal/kg.	3.35	3.29	3.19
NE - Mcal/kg.	2.41	2.36	2.28
Calcium %	0.70	0.70	0.70
Phos.% - available	0.26	0.26	0.26
Phos.% - total	0.60	0.66	0.66
SID Amino acids			
Lys %	1.31	1.31	1.31
Thr %	0.85	0.85	0.85
Met %	0.46	0.46	0.47
Trp %	0.25	0.25	0.25
Ile %	0.75	0.74	0.74

Table 12. (Continued)

Val %	0.87	0.87	0.87
Sodium %	0.18	0.18	0.18
Chlorine %	0.28	0.28	0.28
Magnesium %	0.20	0.30	0.30
Potassium %	0.90	0.90	0.90
Sulfur %	0.23	0.23	0.26

[1]Supplied per kg of basal diet: 10,417 IU of vitamin A as retinyl acetate, 1,458 IU of vitamin D_3 as cholecalciferol, 104 IU of vitamin E as dl-α-tocopheryl acetate, 37.5 mg of niacin as nicotinic acid, 31 mg of pantothenic acid as calcium D-pantothenate, 8 mg of riboflavin as riboflavin, 5 mg of pyridoxine as pyridoxine hydrochloride, 4 mg of vitamin K as 2-methyl-1,4 naphthoquinone-2-sodium bisulfite, 4 mg of folacin as folic acid, 2.5 mg of thiamine as thiamine mononitrate, 0.5 mg of biotin as D-biotin, 0.03 mg of vitamin B_{12} as cyanocobalamin, and 0.17 mg chromium as chromium propionate
[2]Supplied per kg of basal diet: 118 mg of Ca as $CaCO_3$, 51 mg of Zn as $ZnSO_4$, 51 mg of Fe as $FeSO_4$, 15 mg of Mn as $MnSO_4$, 8 mg of Cu as $CuSO_4$, 0.14 mg of I as Ca $(IO_3)_2$ and 0.14 mg of Se as Na_2SeO_3.
[3] Ground dried sludge was ashed through combustion (Table 12 details mineral content).
[4] Sludge was greenhouse-dried and ground (Table 13 details mineral content).

The diets were formulated using the National Swine Nutrition Guide Formulation Software (NSNG, 2010) manufactured at the North Carolina State University Feed Mill Education Unit (Raleigh, NC) and presented in meal form. Representative samples of the three diets per phase were collected and analyzed in duplicate at the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratory for chemical verification.

Pigs were fed with *ad libitum* access to feed and water. Over the four-week period, pigs were weighed weekly, and average daily gain was calculated for each period and overall. Feed additions to the feeders were recorded, and feed remaining after each weekly period was weighed to determine feed disappearance for each period and overall. Feed efficiency was calculated as daily gain divided by daily feed intake for each period and overall. Blood samples were collected on days 14 and 28, and serum was separated by centrifugation at 900 × g for 20 minutes at 20°C. The resulting serum samples were stored at -80°C for subsequent analysis. During the final three days of the study (days 26, 27, and 28), representative fresh fecal samples were collected from each pen, pooled, and stored at -20°C for subsequent processing. The collected samples were dried at 64°C for a minimum of 72 hours until moisture removal was complete. Afterward, the dried samples were ground to a uniform particle size sealed in plastic zip-lock bags, and stored in a climate-controlled room for further analysis of mineral concentrations and apparent total tract digestibility

Chemical analyses

Samples of ashed swine lagoon sludge, dried swine lagoon sludge, and monocalcium phosphate (Table 12) were submitted to Midwest Laboratories Inc. (Omaha, NE) and analyzed in quadruplicate for concentrations of P, Ca, S, K, Mg, Na, Fe, Mn, Cu, and Zn. In the case of dried swine lagoon sludge, the presence of viruses and bacteria might be relevant. While the mineral composition was the primary focus of this study, the microbial safety of dried swine lagoon sludge warrants consideration. Unlike ashed sludge, which undergoes high-temperature combustion that eliminates microbial contaminants, dried sludge is processed through dehydration, which may reduce but not necessarily eliminate bacteria and viruses. Previous studies have demonstrated that lagoon sludge can harbor microbial pathogens (Cardinali-Rezende et al., 2012; Owusu-Twum & Sharara, 2020b). If the sludge is not subjected to sterilization or high-temperature treatment, some of these microorganisms may persist. Since no microbiological analysis was conducted in this study, the potential presence of bacteria and viruses in dried sludge remains uncertain.

Table 13. Analyzed concentrations of minerals in monocalcium phosphate and ashed & dried swine lagoon sludge analyzed in quadruplicate for experiment 2 (as-is basis)

Minerals	**MCP[1]**	**DSLS[1]**	**ASLS[1]**
Sulfur %	0.77 ± 0.11	0.7425 ± 0.10	0.11± 0.01
Phosphorus %	20.43 ± 2.35	4.5225 ± 0.46	9.19 ± 0.62
Potassium %	0.14 ± 0.02	0.39 ± 0.05	0.40 ± 0.03
Magnesium %	0.54 ± 0.02	1.9375 ± 0.24	4.01± 0.30
Calcium %	13.85 ± 2.05	4.59 ± 0.35	7.77 ± 0.62
Sodium %	0.15 ± 0.02	0.1175 ± 0.01	0.14 ± 0.01
Iron ppm	4162.5 ± 619	6760 ± 901	13525 ± 1024
Manganese ppm	369.5 ± 54	726.75 ± 68	1307.5 ± 97
Copper ppm	n.d	1392.5 ± 195	2392.5 ± 206
Zinc ppm	396 ± 63	3055 ± 386	5052.5 ± 374

[1]Each value represents the analyzed concentration of minerals in quadruplicate and its standard deviation. Monocalcium phosphate (MCP), dried swine lagoon sludge (DSLS), ashed swine lagoon sludge (ASLS), no detected (n.d).

Serum samples were submitted to 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).

Calculations and statistical analysis

The statistical analysis for this study was performed using JMP Pro 17 software (SAS Institute Inc). The experimental units were the pens with four pigs each, and they were considered as blocks in a randomized complete block design. The randomization of blocks was based on the initial body weight of pigs, ensuring a balanced distribution across treatments. Body weight and average daily gain data were analyzed with analysis of covariance using initial body weight as the covariate and diet (ASLS, DSLS, and MCP) as factor levels. Feed efficiency, feed intake, and serum P concentrations, were analyzed through an analysis of variance (ANOVA) with block effects included in the model and Tukey HSD for multiple comparisons of treatments. Statistically significant differences were considered at $P \leq 0.05$ with tendencies at $0.05 < P \leq 0.10$.

Results and discussion

Growth performance

During phase 1 (days 0-14), body weight and average daily gain did not differ ($P = 0.47$) between dietary treatments (Table 13), suggesting that ASLS and DSLS, as primary P sources, maintained growth performance comparable to MCP. Findings reported by other researchers (Ryan et al., 2011; Cemin et al., 2024) showed that varying levels of available phosphorus did not impact growth performance in early nursery phases. This suggests that even if dietary available P is reduced between sources, its impact on growth may be difficult to detect in short term effects. Daily feed intake was higher for pigs fed the DSLS diet ($P= 0.006$; 549.19 g/d) compared to those fed the MCP (496.45 g/d) or ASLS (483.04 g/d) diets during Phase 1. As a result, the gain:feed ratio was lower for pigs fed DSLS ($P < 0.001$; 0.66) compared to those fed MCP (0.72) or ASLS (0.73). The increased feed intake observed in pigs fed the DSLS diet may be attributed to diet rejection, resulting in higher feed waste. Analysis of existing literature showed no significant changes in feed intake reported in cases where P availability was lower in the diet (Misiura et al., 2020). This suggests that the observed increase in feed intake in this study is unlikely due to reduced P availability, as there was no consistent impact on intake in those conditions. Overall body weight in Phase 2 was lower in pigs fed the ASLS diet ($P< 0.004$; 20.46 kg) than in those fed

MCP (21.77 kg) or DSLS (21.77 kg) diets, while overall daily feed intake was higher for pigs fed DSLS (P= 0.002; 832.54 g/d) compared to those fed MCP and ASLS diets (770.93 and 750.81 g/d, respectively). A decrease in growth over a 28-days period has been reported with lower levels of dietary available P in pigs (Smith et al., 2023), suggesting that P available from the ASLS diet was likely lower compared to the DSLS and MCP diet. Gain:feed ratio was lower in pigs fed ASLS and DSLS (P<0.001) compared to those fed the MCP diet.

Table 14. Response of growth performance[1] to dietary P from MCP, ASLS, and DSLS sources during phase 1 and phase 2 of the study.

Variable	MCP	ASLS	DSLS	*SE*	*P-value* [2]
Initial BW (kg)	6.99	7.00	6.99	*	<0.001
ADG, P1 (g)	356.21	347.27	371.99	115.62	0.474
ADG, P2 (g)	528.11^{a}	481.26^{b}	528.02^{a}	89.64	0.004
BW, P1 (kg)	12.04	11.84	12.19	1.59	0.467
BW, P2 (kg)	21.77^{a}	20.46^{b}	21.77^{a}	2.51	0.004
ADFI, P1 (g/d)	496.45^{b}	483.04^{b}	549.19^{a}	56.08	0.006
ADFI, P2 (g/d)	770.93^{b}	750.81^{b}	832.54^{a}	60.07	0.002
G:F ratio, P1	0.72^{a}	0.73^{a}	0.66^{b}	0.042	<0.001
G:F ratio, P2	0.68^{a}	0.64^{b}	0.64^{b}	0.021	<0.001

[1]Each value represents the least squares mean of 16 pens with 4 pigs per pen for each treatment group over a 28-day experimental period.
[2]P-value represents the main treatment effect.
Least square means within a row without a common superscript (a, b) are significantly different (P < 0.05).
Average daily gain (ADG), body weight (BW), average daily feed intake (ADFI), gain: feed (G:F), phase 1 (P1), phase 2 (P2).

Serum chemistry

Serum P concentrations on day 14 tended to be lower (P = 0.06) in pigs fed the ASLS diet compared to DSLS but not to MCP (Table 14). Serum Mg concentrations were lower (P = 0.03) in pigs fed the MCP diet compared to those fed the DSLS diet, but not from pigs fed the ASLS diet. Additionally, alanine aminotransferase (ALT) concentrations were lower (P = 0.009) in pigs fed the ASLS diet compared to MCP and DSLS diets. Also, creatine concentrations were higher (P = 0.009) in pigs fed the MCP compared to those with ASLS and the DSLS diets.

Table 15. Response of serum chemistry[1] to dietary P from MCP, ASLS, and DSLS sources in Phase 1 measured on day 14.

Variable	MCP	ASLS	DSLS	*SE*	*P-value*[2]
T.Protein (g/dL)	4.36	4.40	4.45	0.69	0.49
Albumin (g/dL)	2.93	2.89	3.02	0.98	0.55
Globulin (g/dL)	1.42	1.51	1.43	0.31	0.71
P (mg/dL)	7.85	7.46	8.42	1.11	0.06
Ca (mg/dL)	10.46	10.41	10.17	0.75	0.52
Mg(mEq/L)	1.52^{b}	1.63^{ab}	1.68^{a}	0.15	0.03
K(mEq/L)	5.39	5.20	5.21	0.48	0.46
Na(mEq/L)	142.94	142.82	142.41	2.65	0.85
Na/K ratio	26.69	27.82	27.82	2.71	0.42
Cl (mEq/L)	101.30	100.09	100.85	1.77	0.19
BUN (mg/dL)	7.25	6.16	7.02	2.57	0.48
AST (IU/L)	45.81	52.08	36.83	17.37	0.08
ALT (IU/L)	34.43^{b}	27.78^{a}	33.23^{b}	3.95	0.009
GGT (IU/L)	29.88	33.26	31.49	7.67	0.28
SDM	18.43	18.26	16.61	2.96	0.21
Glucose	113.62	106.22	114.64	12.20	0.14
Creatine	0.69^{a}	0.60^{b}	0.60^{b}	0.082	0.009
Alk phosp.	314.53	304.47	325.07	60.44	0.68

[1]Each value represents the least squares mean of 16 pens per treatment group on day 14.
[2]P-value represents the difference between treatments
Least square means within a row without a common superscript (a, b) are significantly different (P < 0.05).
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 phosphatase48 (AP), gamma-glutamyl transferase (GGT), and symmetric dimethylarginine (SDMA).

On day 28, serum P concentrations in pigs fed the ASLS diet were lower ($P < 0.001$) compared to MCP and DSLS diets. When serum P was used as criterion to assess P bioavailability, results suggest that P in ashed sludge may be less available then the other two sources. This reduced availability could be attributed to the thermal processing used to produce ASLS, which may have altered the chemical structure and solubility of phosphorus. A recent review by Luo et al. (2024) on P recovery from sewage sludge has shown that thermal treatments can influence P speciation, often leading to the formation of more crystalline or less soluble compounds with reduced bioavailability. However, most studies have focused on plant systems, and limited research has evaluated the effects of thermal processing on P bioavailability in animals. Further investigation is needed to determine how different thermal treatments impact P digestibility, absorption, and utilization in livestock. Serum Mg concentrations tended to be lower ($P = 0.06$) in pigs fed MCP diets (1.66 mEq/L) compared to ASLS (1.81 mEq/L) and DSLS (1.74 mEq/L). Unlike day 14, no significant differences were observed in ALT concentrations between dietary treatments on day 28. Creatine concentrations were not affected by treatments on day 28 (Table 15).

Table 16. Response of serum chemistry[1] to dietary P from MCP, ASLS, and DSLS sources in phase 2 (d28).

Variable	MCP	ASLS	DSLS	*SE*	*P-value*[2]
T.Protein (g/dL)	5.08	5.11	5.13	0.27	0.87
Albumin (g/dL)	3.45	3.40	3.42	0.20	0.82
Globulin (g/dL)	1.62	1.71	1.71	0.24	0.59
A/G ratio	2.16	2.02	2.06	0.33	0.56
P (mg/dL)	9.16[a]	7.31[b]	9.35[a]	0.94	<.001
Ca (mg/dL)	11.65	11.59	11.24	0.64	0.29
Mg(mEq/L)	1.66	1.81	1.74	0.16	0.06
K(mEq/L)	5.85	5.72	5.33	0.53	0.07
Na(mEq/L)	145.31	145.62	144.92	3.00	0.85
Na/K ratio	25.12	25.71	27.48	2.41	0.08
Cl (mEq/L)	100.93	101.38	100.57	1.71	0.50
BUN (mg/dL)	8.42	7.95	8.23	2.77	0.90
AST (IU/L)	32.63	36.92	31.78	8.29	0.25
ALT (IU/L)	36.11	34.82	34.18	5.71	0.71
GGT (IU/L)	27.19	30.27	27.59	5.97	0.36
SDM	16.43	17.70	15.63	3.48	0.34
Glucose	128.35[a]	121.74[b]	127.36[ab]	6.81	0.04
Creatine	0.67	0.63	0.67	0.12	0.51
Alk phosp.	308.88	332.86	296.29	61.36	0.33

[1]Each value represents the least squares mean of 16 pens per treatment group on day 28.
[2]P-value represents the difference between treatments
Different superscripts indicate significant differences based on Tukey's multiple comparison analysis
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 phosphatase48 (AP), gamma-glutamyl transferase (GGT), and symmetric dimethylarginine (SDMA).

Economic assessment

Periodic removal of sludge from lagoons is an essential practice in all swine operations to maintain proper waste management and prevent the accumulation of excess nutrients and pathogens in lagoons. Swine lagoons may contain harmful pathogens like E. coli and Salmonella, posing health risks through direct on-farm contact or off-farm exposure (Cordero et al., 2010). Pathogens may contaminate groundwater via seepage, enter surface water through runoff or overflow, or become airborne during spray irrigation. Proper lagoon management is essential to minimize these risks and protect both farmworkers and surrounding communities (Hill & Sobsey, 2003). Over time, swine farmers have developed a variety of innovative strategies and pathways to manage sludge, tailoring their approaches to fit specific operational needs, environmental conditions, and regulatory requirements (Binder et al., 2023). These alternatives range from conventional methods, such as applying sludge as fertilizer on agricultural land, to more advanced techniques like composting. Each pathway offers unique benefits and challenges. Applying sludge as fertilizer offers a method to recycle nutrients back into the soil, reducing reliance on synthetic fertilizers and promoting sustainability. However, challenges arise due to the nutrient composition of the sludge, especially the high concentrations of heavy metals such as Cu, Zn, and Mn. These metals can limit the amount of sludge that can be safely applied to fields or as a dietary ingredient for pigs. Excessive accumulation of these metals, along with salts and other elements, can lead to soil toxicity, negatively impacting plant health and soil quality over time (Grobelak et al., 2024). The application of minerals in agricultural soils can directly affect the mineral content of harvested crops, potentially impacting both crop quality and food safety. Over time, repeated pig manure applications have been shown to increase the concentration of these heavy metals in the soil. A study on rice cultivation revealed that this buildup of heavy metals in soils is closely correlated with elevated levels of the same metals in rice grains (Lan et al., 2022). This suggests that the heavy metals introduced through pig manure are absorbed by the plants and accumulate in the edible parts, raising concerns about long-term soil health and the potential for heavy metal contamination in the food supply. Dietary requirements of Zn and Cu for pigs are very low. However, when supplied at high concentrations (100 to 250 ppm for Cu and 2000 to 3000 ppm for Zn) growth performance is positively influenced, especially in young pigs (Jacela et al., 2010). It is important to highlight that the response to high levels of these minerals decreases with increasing age and with longer periods of administration. In pigs, prolonged dietary intake of Cu exceeding the high levels suggested can lead to toxicity, resulting in red blood cell hemolysis, which manifests as jaundice and tissue necrosis (Espinosa & Stein, 2021).

Precise diet formulation to provide accurate dietary needs and lagoon treatment systems offer effective solutions for reducing heavy metal concentrations in swine waste. Studies suggest that lagoon systems, particularly one- or two-stage systems, are effective in reducing heavy metal concentrations, with reductions of 95%, 36%, 24%, and 24% for Cu, Zn, Mn, and Na, respectively for effluent from a single lagoon (Overcash et al., 1978). A recent study by Patil (2023) compared the economic value of five different pathways for utilizing swine lagoon sludge. The first and most common method involved conventional sludge management through agitation, pumping, and transporting the sludge for land application as fertilizer. The second pathway explored an alternative approach where the sludge is agitated, pumped into a dewatering system, and bagged for easier handling. The third pathway focused on composting the sludge to create an organic soil amendment. The fourth and fifth pathways involved sludge removal using an excavator; in the fourth method, the sludge was spread directly after removal, while in the fifth, it was dried first before being spread, enhancing its ease of application and storage. Of these, the economic assessment was focused on the first and fifth pathways. The estimated cost of the fifth pathway (216$ /Mt of dried sludge) was used to compare and create a sixth pathway for the utilization of sludge as a mineral supplement for swine diets (Figure 8).

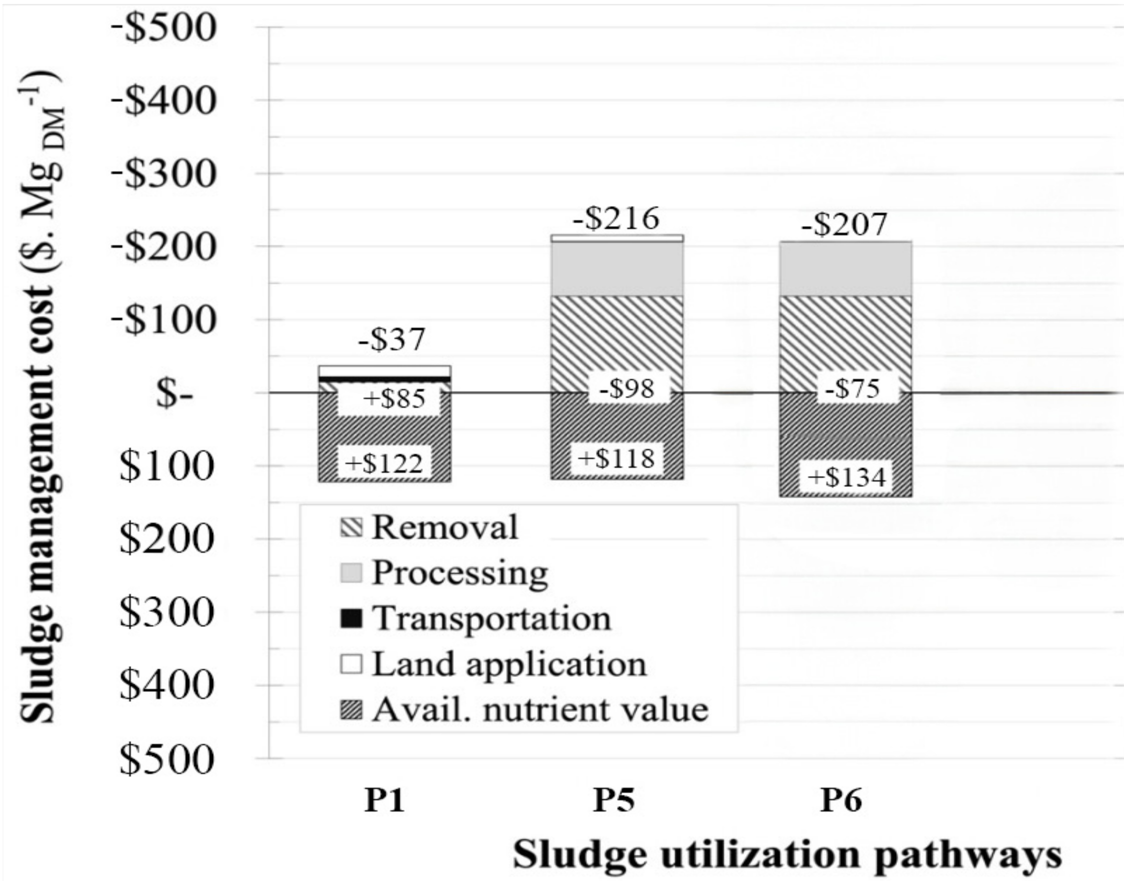


Figure 8. Economic comparison between sludge management practices

[a]P1: Agitation>pumping>land application, P5: Excavation>drying>land application, P6: Excavation>drying>diet inclusion.

[b]Labels shown for each pathway indicate: pathway cost, end-product value, and net-cost on a dry-basis ($. Mg^{-1})

The cost of using sludge as a feed ingredient was estimated at $207 per metric ton (Mt) of sludge. This value reflects a reduction of $9 from 216$ estimated per metric ton from the 5th pathway, which represents the cost of land application—a step that would be avoided if the sludge were repurposed as a feed ingredient. To determine the final cost per pound of available P, concentrations from analyzed monocalcium phosphate (MCP) and dried sludge (DSLS) samples were used to determine the total product mass required (4.89 lbs of MCP and 22.12 lbs of DSLS per lb of P). These values were then adjusted based on bioavailability data from McAuley (2022), who reported a relative bioavailability value of 67.1 % for DSLS, resulting in a bioavailability equivalency of 1:1.5 for MCP to DSLS. The calculated costs per pound of MCP and DSLS ($0.41 and $0.093, respectively) were multiplied by their respective sources, yielding the final cost per pound of available phosphorus (Table 17).

Table 17. Estimated cost $ of available P in MCP and DSLS

	MCP	**DSLS**	**ASLS**
Bioavailable P, %	100 (standard)	67.1	68.9
Concentration of P, %	20.43	4.52	9.16
Cost $/ lb of product	0.41	0.093	*
Cost $ / lb of P available	2.00	3.08	*

The net cost of P recycled from dried swine lagoon sludge in this example calculation is 54% more expensive than the cost of P provided by monocalcium phosphate (MCP). However, this estimate is influenced by several factors, including the specific management options used for sludge. For instance, the conventional and cheapest pathway of land application (Pathway 1) presents significant limitations. Many farms are concentrated in specific regions, leading to limited available land for sludge application. As a result, transportation costs increase substantially with every additional kilometer traveled to transport the material to alternative locations (Figure 9), creating a linear relationship between net sludge utilization cost and distance from the source. In contrast, the increase in transportation costs per kilometer for dried sludge is considerably lower (Pathway 5), due to its reduced volume and weight. Drying the sludge decreases its volume by approximately 90% or more. This reduction in volume leads to a flattening of the cost-distance relationship, offering a substantial advantage by minimizing the number of loads required and thereby reducing fuel consumption, labor, and overall transport expenses (Overcash et al., 1978). These logistical challenges further drive up the cost of recycling phosphorus from sludge.

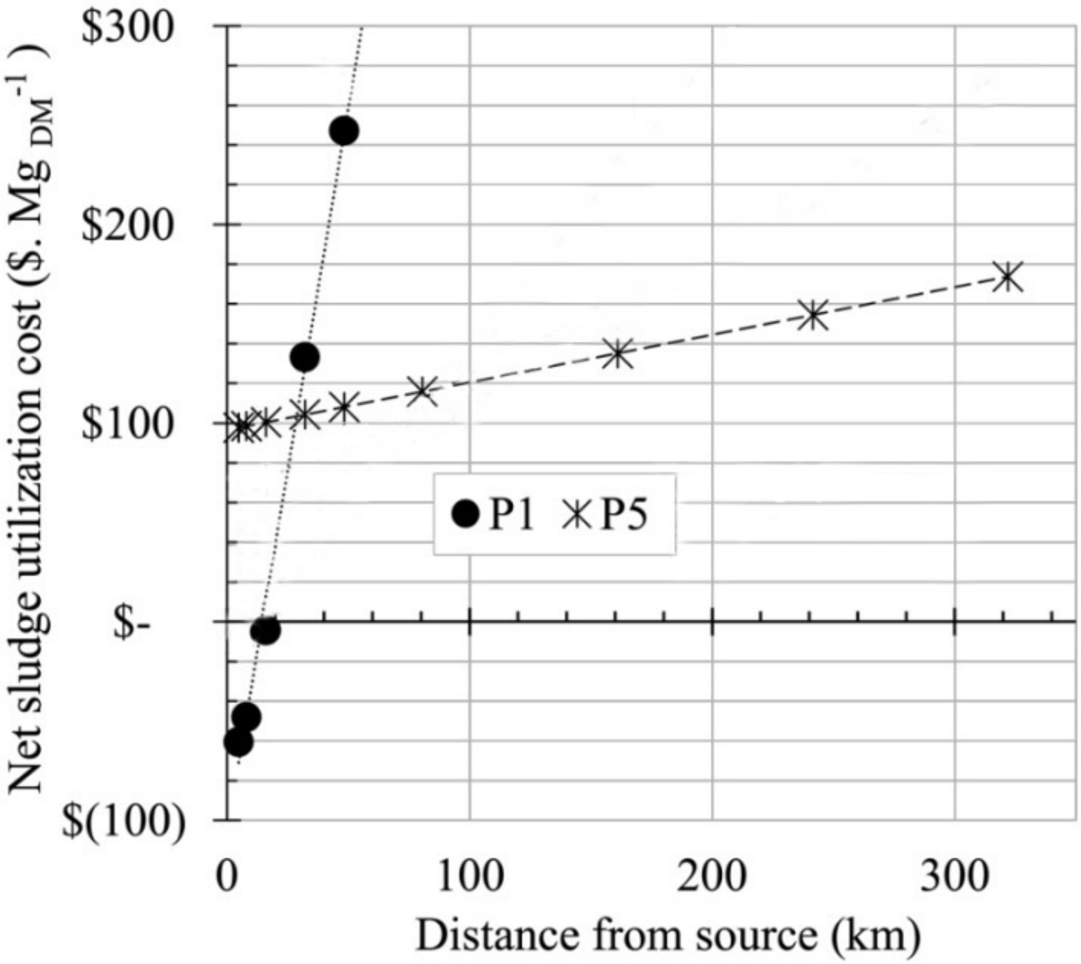


Figure 9. Estimated increase in sludge utilization cost in ($) per megagram of dried sludge (1 Mg = 1,000 kg) with increasing distance from source (Patil, 2023)

These logistical and operational constraints must be carefully considered when calculating the cost of using sludge as either a fertilizer or a mineral supplement in animal diets. Each farm faces unique challenges based on location, infrastructure, and resource availability, meaning that decisions regarding the use of recycled phosphorus must be tailored to each operation's specific circumstances.

The economic value of DSLS as a P source should be viewed as an additional revenue stream that offsets a portion of sludge management costs rather than fully covering removal expenses or generating net profit (Figure 8, pathway 6). Since sludge removal is necessary regardless of its end-use, incorporating DSLS into animal feed provides financial benefits by reducing the need for purchased P. When subtracting the equivalent value of monocalcium phosphate (MCP) at $2.00 per lb of available P, the adjusted net cost of sludge management when DSLS is used as a feed ingredient becomes:

$$3.15 - 2.00 = \mathbf{1.13}\ \boldsymbol{USD\ per\ lb\ P\ available}$$

This value represents the net cost of removing sludge from the lagoon to maintain compliance. Converting this cost from units of available P back to cost per Mt DM:

Net lagoon sludge cleaning cost (feed ingredient use)

$$1.13\left(\frac{USD}{lb\ P\ available}\right) \times 0.671\left(\frac{lb\ P\ available}{lb\ P}\right) \times\ 0.0452\left(\frac{lb\ P}{lb\ DM}\right) \times\ 2{,}204.6\left(\frac{lb}{Mt}\right)$$

$$= \mathbf{75.6}\ \boldsymbol{USD\ per\ Mt\ DM}$$

However, if DSLS is used as a fertilizer instead of a feed ingredient, the net sludge management cost increases. Without the benefit of phosphorus replacement in feed, the net cost for sludge removal remains higher:

Net lagoon sludge cleaning cost (fertilizer use)

$$= \mathbf{98.0}\ \boldsymbol{USD\ per\ Mt\ DM}$$

This comparison highlights that utilizing DSLS in swine diets reduces the net sludge management cost to $75.6 per Mt DM, compared to $98.0 per Mt DM when used as a fertilizer. This cost reduction occurs because, when DSLS is included in feed, its value as a P source offsets part of the sludge removal expense. Despite the economic costs, it is essential to prioritize proper sludge management practices. Effective management not only ensures the sustainability of phosphorus recycling but also minimizes the potential environmental impact, helping to prevent nutrient overloading and contamination of surrounding ecosystems. Therefore, while the economic feasibility of using sludge as a phosphorus source can vary, the long-term environmental and operational benefits may still make it a viable alternative for many farmers.

Conclusions

This study evaluated ashed swine sludge (ASLS) and dried swine lagoon sludge (DSLS) as P sources in nursery pig diets. Both ASLS and DSLS supported average daily gain (ADG) in early Phase 1, but prolonged ASLS use may negatively impact body weight. Feed intake was higher in pigs fed DSLS, reducing the gain-to-feed ratio, while serum P concentrations in Phase 2 were lower in pigs fed ASLS, suggesting reduced P bioavailability. Phosphorus in ASLS appears less available than in DSLS and monocalcium phosphate (MCP). The estimated bioavailability of ASLS (68.7%) may be less reliable due to the loss of the anchor point in the slope ratio. Economically, P from DSLS costs 1.45 times more than MCP, though sludge management practices heavily influence this conclusion. However, this estimate is influenced by sludge management strategies. The land application presents significant limitations due to regional farm concentrations and limited available land, which increases transportation distances and costs. Economic assessment indicated that incorporating DSLS into animal diets offers a greater financial advantage over fertilizer application. Under the drying sludge pathway, the ability to recover its value as a dietary P source reduces the net sludge management cost to $75.6 per Mt DM, compared to $98.0 per Mt DM for fertilizer use. These economic values are not universally applicable but can serve as a reference for calculating costs specific to individual swine operations. Further research is needed to optimize the use of ASLS and DSLS in swine diets.

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