Effects of 25-Hydroxycholecalciferol or Phytase on Calcium, Phosphorus, Amino Acids and Energy Digestibility in Growing Pigs

By

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Dissertation

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Abstract

Four experiments were conducted to test a series of formulated hypotheses regarding vitamin D and microbial phytase in pig diets. In experiment 1, the hypothesis was that calcifediol [**25(OH)D_3**] and microbial phytase have additive effects on the standardized total tract digestibility (**STTD**) of Ca and P, serum bone biomarkers, and plasma vitamin D_3 metabolites when fed to growing pigs. No additive effects of the 2 additives were observed, but inclusion of microbial phytase increased STTD of Ca and P ($P < 0.001$). Addition of 25(OH)D_3 tended to increase STTD of Ca and P only in the absence of phytase (interaction; $P < 0.10$). Serum osteocalcin increased with inclusion of either phytase or 25(OH)D_3 ($P < 0.05$), but there were no changes in other bone biomarkers. Plasma 25(OH)D_3 and 24,25$(OH)_2D_3$ increased with 25(OH)D_3 and/or phytase ($P < 0.05$), whereas plasma 1,25$(OH)_2D_3$ decreased with phytase ($P < 0.001$). It was concluded that phytase and 25(OH)D_3 increased Ca and P digestibility and serum osteocalcin, and 25(OH)D_3 improves vitamin D_3 status, but microbial phytase and 25(OH)D_3 have no additive effects. In experiments 2, 3, and 4, the hypothesis was that graded levels of phytase 0, 500, 1,000, 2,000, and 4,000 phytase units (**FTU**) per kilo, increases apparent total tract digestibility (**ATTD**) of energy, the STTD of Ca and the apparent ileal digestibility (**AID**), and standardized ileal digestibility (**SID**) of amino acids in three different sources of soybean meal (**SBM**) when fed to pigs. Results in experiment 2 demonstrated that source of SBM had no influence on digestible energy (**DE**) and metabolizable (**ME**) in the corn-SBM diets or in the SBM ingredients. Phytase supplementation increased (quadratic, $P < 0.01$) ATTD of dry matter and ATTD of gross energy (**GE**) also increased (linear, $P \leq 0.05$). Consequently, DE and ME of diets increased (quadratic, $P < 0.01$ and $P < 0.05$, respectively), and DE:GE increased (linear, $P < 0.05$) with increasing phytase in diets. Likewise, ATTD of GE in SBM increased (linear, $P <$

0.01) and DE and ME in SBM increased (quadratic, $P < 0.01$ and $P < 0.05$, respectively) as phytase inclusion increased. The conclusion was that microbial phytase improves energy utilization in SBM and ME in SBM is increased by 40 to 50 kcal/kg, independent on the sources of SBM. For experiment 3, source of SBM did not affect ATTD or STTD of Ca in diets. However, source of SBM affected ($P < 0.05$) ATTD and STTD of P and the ATTD of Cu, Mn, and Zn. Increasing phytase increased ($P < 0.05$) absorbed Ca and P as well as ATTD and STTD of Ca and P in diets. Likewise, calculated digestibility of Ca in SBM increased (linear, $P < 0.01$; quadratic, $P \leq 0.05$) as phytase increased from 0 to 4,000 FTU/kg. For experiment 4, Soybean meal source had limited effects on ileal digestibility of crude protein (**CP**) and amin acids (**AA**), with only minor differences observed for dry matter, Trp, CP, Lys, and Gly. In contrast, increasing dietary phytase linearly increased ($P < 0.001$) the AID and SID of CP and all indispensable and dispensable AA. In conclusion, phytase improved protein and amino acid digestibility independent of soybean meal source, indicating that the response to phytase was consistent across sources.

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Dedication

This thesis is dedicated to my mom, and to my 2021 self. I know how dark and painful it was to face my mom's illness and to know that you would lose her soon, while struggling with your sense of vocation. I want to tell you: thank you for keeping your faith that one day you would feel grateful for everything.
You worked hard but trust me—you will feel that everything is worth it when your soul, your heart, and your vocation are aligned. Life will give you a sense of well-being that you never imagined.

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Chapter 1: Introduction

Accurate diet formulation and sustainability are inseparably tied to the growing demand for high-quality animal protein (Akintan et al., 2024). Efficient use of feed ingredients—that inherently require natural resources and contribute to the carbon footprint—places increasing pressure on the livestock industry to enhance both accuracy and sustainability in diet formulation (Henchion et al., 2017). The principal feed ingredients for animals are plant-based and contain phytate which is the primary storage form of P. The P bound to phytate is largely indigestible for pigs. In addition, the phytate in the diets binds Ca, starch, amino acids, and minerals reducing digestibility (Selle et al., 2000; Humer et al., 2014; Espinosa et al., 2022). Therefore, exogenous phytase is often included in swine diets to release P from phytate, minimizing the chelation of other minerals and nutrients thereby reducing the need for inorganic P addition, and overall reducing the cost of the diets (González-Vega et al., 2015). Ca is an important macro mineral that is essential for proper performance, growth, and production. Even so, it is inexpensive nutrient compared with P and amino acids, and is often used as a filler in diets. However, in the formulation of pig diets, it is necessary to consider an appropriate Ca:standardized total tract digestibility (**STTD**) of P ratio to ensure adequate absorption and utilization of both minerals, while avoiding excesses or deficiencies. This is important because dietary Ca influences the digestibility of P, among other nutrients; implementation of STTD Ca and P values is needed to obtain values that are additive in mixed diets and therefore increase accuracy in diet formulation (González-Vega and Stein, 2014; Lee et al., 2023, Stein 2024).

Nutritional matrix values for precise diet formulation using soybean meal (**SBM**) and graded levels of phytase are also needed to estimate the contribution to the STTD of nutrients, including energy, amino acids, and Ca released from SBM. These values should account for the

"extra-phosphoric" effects of microbial phytase superdosing and be independent of the SBM source in diets for growing pigs (Velayudhan et al., 2025).

In addition to dietary strategies such as phytase inclusion, physiological regulation of Ca and P absorption also plays a critical role. To maximize the absorption of Ca and P, the activated form of vitamin D_3 must bind to the vitamin D receptor in the small intestine. Activation of dietary vitamin D_3 requires hydroxylation at the 1 and the 25 positions are needed, with results in 1,25 $(OH)_2D_3$, which is the active form of vitamin D_3. It is possible vitamin D_3 metabolites such $25(OH)D_3$ offer benefits over vitamin D_3 in diets for growing pigs, and that this metabolite results in improved vitamin D status of the pig (Lee et al., 2022). However, it is not known if effects of $25(OH)D_3$ and microbial phytase are additive in diets for pigs.

Therefore, four experiments were conducted to determinate effects of graded levels of phytase supplementation on the digestibility of energy, amino acids, and Ca in SBM. The second hypothesis was that effects of microbial phytase and $25(OH)D_3$ in diets fed to growing pigs are additive.

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Chapter 2: Effects of vitamin D_3 metabolite and phytase on digestibility of energy and nutrients by growing pigs: A literature review

Introduction

Calcium and P are essential minerals in pig nutrition, playing critical roles in skeletal development forming the primary components of bone tissue, metabolic functions, in growing pig performance (Gonzalez-Vega and Stein., 2014; Lautrou et al., 2021). Beyond bone health, P is important for energy metabolism through adeno triphosphate, whereas Ca is needed for muscle contraction, nerve function, and enzyme activation (Stipanuk and Caudill, 2019). Results of recent research have improved the understanding about utilization of both minerals (Gonzalez-Vega and Stein., 2014). Excessive Ca in diets negatively impacts animal growth performance due to interactions with the absorption of other nutrients, including P. Cereals and oilseed meals contain high levels of P bound to phytic acid, but this form is poorly digested by pigs, resulting in increased P excretion, which may contribute to eutrophication of water and soil (Simons et al., 1990). These concerns have triggered research over the past decades focused on increasing the availability of P from phytate by adding exogenous phytase to pig diets. As is the case with other nutrients, accurate provisions of Ca and P in pig diets depend on determining the digestibility of the nutrients in dietary ingredients (Lagos et al., 2021). Cholecalciferol (Vitamin D_3) is also important for maximizing the effectiveness of dietary Ca and P, especially by supporting absorption of Ca and P from the small intestine and resorption of Ca and P in the kidneys. Vitamin D_3 is produced in the skin through ultraviolet irradiation of 7-dehydrocholesterol, but in animal diets synthetic vitamin D_3 is provided (NRC, 2012).Vitamin D_3, is a pre-hormone that is biologically inert and must be hydroxylated in the liver and then in the kidney, to form 25 hydroxycholesterol [**$25(OH)D_3$**] and 1,25 dihydroxycholecalciferol [**$1,25(OH)_2D_3$**], respectively

(Zempleni et al., 2007). The active form, $1,25(OH)_2D_3$, as well as calcitonin and parathyroid hormone (**PTH**) are needed to control blood Ca and P levels (Jones et al., 1998; Combs and McClung, 2017). Vitamin D_3 is also important for other physiological systems including prevention of myopathies, insulin resistant protective factors, and immunity (DeLuca, 2004; Zempleni et al., 2007).

Vitamin D_3 history

At the start of the nineteen century, the understanding that epidemic diseases could be derived from nutritional deficiencies resulted in an interest in the study of protein, carbohydrates, fat, and minerals (Semba, 2012). The confinement of animals used as experimental models provided insights into nutritional requirements and deficiencies. In 1906, it was discovered that a complete diet with protein, starch, cane sugar, lard, and minerals, was insufficient to sustain normal growth of young rats (Hopkins, 1912). Subsequently, it was elucidated that the deficiency was not attributable to a “nutritive error”, but that some “accessory factors” essential for sustaining life, were absent in that base diet (Hopkins, 1912). Four years later, those “accessory factors” would be designated as “vitamins” (Semba, 2012; Souganidis, 2012).

In 1913, it was discovered that supplying the ether soluble portion of egg or butter fat to the diet is necessary for normal growth by young animals (McCollum and Davids, 1913). Animals fed purified diets developed xerophthalmia (eye disease), which could be efficiently cured using butter fat or cod liver oil. It was, therefore, determined that butter fat and cod oil contained an unknown factor called "fat-soluble A" (McCollum and Davids, 1913; DeLuca, 2016; Jones, 2022). In 1919, the British doctor Edward Mellanby fed puppy dogs oatmeal and kept them indoors, simulating the nourishment and lifestyle of children with rickets (O’Riordan and Bijvoet, 2014). The diet and confinement resulted in severe rickets in the dogs, but Mellanby

cured them using cod liver oil. It was, therefore, logical to conclude that the "factor A" contained in the cod liver oil that cured xerophthalmia also had another activity that allowed it to cure rickets (DeLuca, 2004; Jones, 2022). Concurrent with those discoveries, Oscar Huldshinsky and Harriet Chick, exposed some children with rickets to the sun light and UV light, curing them also (Chick, 1976; O'Riordan and Bijvoet, 2014).

By bubbling oxygen through heated cod liver oil, the vitamin A activity was eliminated, but even without vitamin A, the cod liver oil cured rickets, leading to the discovery of vitamin D (McCollum et al., 1922; O'Riordan and Bijvoet, 2014; Jones, 2022). Thus, it was determined that sunlight exhibited the same curative ability for rickets as vitamin D; this dichotomy lead to research with irradiated animals, food, and animal cages, which resulted in animals being cured for rickets, or prevented rickets from developing (Steenbock and Black, 1924). Results also demonstrated that UV light irradiation converts an inactive lipid into an active antirachitic substance, which eventually resulted in the use of dietary vitamin D and its inclusion in food (Jones, 2022).

In 1932, vitamin D_2 was isolated from an irradiation mixture of ergosterol, which is a sterol in cell membranes, fungi, and protozoa (Askew et al., 1931; DeLuca, 2014).). In 1935, 7-dehydrocholesterol was isolated and two years later, vitamin D_3 was identified as the natural product of UV light irradiation in the skin (Holick et al., 1977; Wolf, 2004). Between 1930 and 1960, research revealed that vitamin D is connected to PTH and calcitonin, both Ca and P related hormones (Jones, 2022).

Between 1950 and 1970 advances were made in the discovery of vitamin D metabolism (Kodicek, 1974: DeLuca 2004; Jones, 2022), and it was discovered that vitamin D_3 is in fact converted to more biologically active forms *in vivo* than *in vitro* (DeLuca, 2016). To determine

the final active form of vitamin D, radiolabeled vitamin D_3, was administrated to chickens and the metabolite was isolated in intestinal mucosa in a pure form and the structure was identified as $1,25(OH)_2D_3$ (Holick et al., 1971; Jones, 2022).

A protein receptor for $1,25(OH)_2D_3$, located on the external membranes of the intestinal cells, was also discovered and this protein receptor is now known as the vitamin D receptor (Zempleni et al., 2007). The signal transduction mechanisms of classical vitamin D receptor, which binds $1,25(OH)_2D_3$ in several target tissues, indicates that vitamin D has multiple functions in the body (Brumbaugh and Hausser, 1973; Kresge et al., 2006; Combs and McClung, 2017; Jones, 2022).

The vitamin D receptor selectively binds $1,25(OH)_2D_3$ and controls the expression of selected genes in target cells involved in Ca and P homeostasis (Zempleni et al., 2007). Vitamin D is important for mineral regulation in several tissues including the intestines, kidney, placenta, eggshell gland, and bone (Combs and McClung, 2017). The active metabolites of vitamin D_3 have been synthetized (Leyssens et al., 2014). With the increased interest in the digestibility of energy and nutrients, vitamin D_3 metabolites have also been developed for livestock feed and several vitamin D_3 metabolites may increase the digestibility of Ca, P, and energy in gestating sows, apparently because of an insufficient conversion of cholecalciferol to the active form of vitamin D_3 (Lee et al., 2022).

Vitamin D_3 activation and metabolism

Vitamin D is the generic descriptor for a group of steroid compounds critical for bone and mineral metabolism (Jones et al., 1998), and vitamin D is important in prevention and treatment of rickets and osteomalacia (Combs and McClung, 2017; Pilz et al., 2019). The main forms of Vitamin D are ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3); both are

pre-hormones that are subsequently activated by hydroxylation (McDowell, 1989; Hurts et al., 2020). Vitamin D_2 can be produced synthetically by the photolysis of plant sterols, but has a moderate to low bioactivity, and is mostly used in food fortification and supplementation for humans (Combs and McClung, 2017). Vitamin D is supplied in animal diets as vitamin D_3, which is absorbed in the small intestine with the aid of bile salts after entering the enterocyte by micelle-dependent diffusion (McDowell, 1989; NRC, 2012; Dawson-Hughes et al., 2015). Like other lipidic vitamins, vitamin D is absorbed in mammals via lymphatic circulation binding to the carrier vitamin D-binding protein (Zempleni et al., 2007). Once in the bloodstream, vitamin D_3 reaches the liver to undergo a hydroxylation at the carbon 25 position, by the 25-hydroxylase enzyme forming $25(OH)D_3$ (DeLuca, 2014). The $25(OH)D_3$ cytochrome P450 enzyme, 25-hydroxylase, is poorly regulated (Bikle, 2021). After the liver, $25(OH)\ D_3$ is bound to the carrier vitamin D-binding protein and albumin and circulates in the bloodstream or serum, before being transported to the kidneys (Combs and McClung, 2017). If there is a lack of Ca or P, the $25(OH)D_3$ is hydroxylated by the 1α-hydroxylase enzyme at the 1α carbon position to form $1,25(OH)_2D_3$ (Tanaka and DeLuca, 1973). This conversion is tightly regulated: low plasma Ca (via increased PTH) and low plasma P stimulate 1α-hydroxylase activity, whereas high circulating $1,25(OH)_2D_3$ suppresses it (Takada and DeLuca, 1973, Combs and McClung, 2017). The active form of vitamin D_3, $1,25(OH)_2D_3$ is rapidly hydroxylated by 24-hydroxylase to produce $1,24,25(OH)_3D_3$ (Combs and McClung, 2017). The 24-hydroxylase enzyme also regulates $25(OH)D_3$ producing the inactive form $24,25(OH)_2D_3$, but the enzyme has greater affinity for $1,25(OH)_2D_3$. The conversion of active $1,25(OH)_2D_3$ to its inactive form prevents excessive active vitamin D_3 in circulation, which prevents hypercalcemia and hyperphosphatemia (Bikle, 2021; Figure 2.1)

Vitamin D_3 metabolites: measurement and practical relevance

The biological common forms of vitamin D are $25(OH)D_3$ and the biologically active form $1,25(OH)_2D_3$, which are often measured in clinical practice (Hurst et al., 2022). Whereas $25(OH)D_3$ remains the analyte of choice for the diagnosis of vitamin D deficiency, analysis for $1,25(OH)D_3$ is only recommended in a few conditions with a dysregulated vitamin D metabolism, and LC–MS/MS is the preferred method for the measurement of all vitamin D related analytes as it offers high sensitivity and specificity (Makris et al., 2020). In particular, $25(OH)D_3$ and $24,25(OH)_2D_3$ can accurately be measured with this technology. When interpreted together, they provide a measure of vitamin D metabolism beyond the analysis of $25(OH)D_3$ alone. Determination of vitamin D binding proteins and free and bioavailable $25(OH)D_3$ is compromised by unresolved analytical issues, lacking reference intervals and insufficient clinical data (Alonso et al., 2023; Zhu et al., 2021). Therefore, future research activities should focus on analytical standardization and exploration of their clinical value.

$25(OH)D_3$ determination for vitamin D_3 status

The most abundant vitamin D metabolite in the blood is $25(OH)D_3$, which includes $25(OH)D_2$, derived from ergocalciferol and $25(OH)D_3$ derived from cholecalciferol (Alonso et al., 2023). The serum circulating $25(OH)D_3$ measurement is recommended to evaluate vitamin D status, because $25(OH)D_3$ is associated with clinical outcomes such as bone mineralization, fracture risk, and cardiovascular events (Hermann et al., 2017). Additionally, its levels in the blood is around thousand times greater (8-165 nmol/L) compared with $1,25(OH)_2D_3$ (50 to 150 pmol/L; Makris et al., 2020). However, measuring $25(OH)D_3$ has limitations, because low $25(OH)D_3$ levels may not always give the expected clinical signs of deficiency and its association with bone mineral density varies depending on ethnicity, geographical location, and season. These factors

increase analytical variation among assays and contribute to discrepancies in reported results (Ladang et al., 2024).

24,25(OH)$_2$D$_3$ determination for vitamin D$_3$ status

24,25(OH)$_2$D$_3$ is formed when 25(OH)D$_3$ is hydroxylated at the 24th carbon position by the enzyme 24-hydroxylase. This reaction is part of the vitamin D catabolic pathway, meaning it is involved in breaking down and inactivating vitamin D. Although it has been considered an inactive degradation product, 24,25(OH)$_2$D$_3$ may have biological functions, that involve bone repair and growth and chondrocyte differentiation, which is important in cartilage development (Boyan et al., 2001). Under normal physiological conditions, serum levels of 24,25(OH)$_2$D$_3$ typically represent about 10% of the concentration of 25(OH)D$_3$. These two metabolites are generally strongly correlated. As a result, measuring 24,25(OH)$_2$D$_3$ alone does not usually offer additional clinical insight beyond what is provided by assessing 25(OH)D. However, the combined measurement of both metabolites is valuable in detecting individuals with impaired 24-hydroxylase enzyme activity (Alonso et al., 2023).

1,25(OH)$_2$D$_3$ active form measured as vitamin D status

The major hormonally active form of vitamin D, 1,25(OH)$_2$D$_3$, is measured by a competitive binding assay using HPLC, and an LC/MS/MS assay, which may distinguish between the D$_2$ and D$_3$ forms of 1,25(OH)$_2$D$_2$, and 1α,25(OH)$_2$D$_3$ (Tuckey et al., 2019). Although, 1,25(OH)$_2$D$_3$ is the biologically active form of vitamin D, it is not the ideal measure for vitamin D status because the half-life of circulating 1,25(OH)$_2$D$_3$ is only 4-6 hours. Circulating concentrations of 1,25(OH)$_2$D$_3$ are approximately 1,000-fold less than those of 25(OH)D$_3$ (Alonso et al., 2023). In vitamin D deficiency, intestinal Ca absorption is impaired, resulting in a reduction in ionized Ca levels (Areco et al., 2020). This decrease is sensed by calcium-sensing receptors in the

parathyroid glands, which respond by upregulating PTH secretion, which is important for Ca homeostasis by enhancing renal Ca reabsorption, promoting bone resorption, and stimulating the renal conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ (Hurst et al., 2022). Consequently, individuals with vitamin D insufficiency or deficiency may maintain normal or even elevated serum levels of $1,25(OH)_2D_3$. Therefore, measurement of $1,25(OH)_2D$ is not a reliable marker for assessing overall vitamin D status (Holick, 2010).

Phytate and phytase in pig nutrition

Phytate (myo-inositol hexakisphosphate; **IP_6**), the salt form of phytic acid, represents 1 to 3% of the dry weight of most cereal grains, oilseeds, and legumes (Cosgrove, 1980; Reddy et al., 1982). In plant-based feed ingredients, P is predominantly stored in this form, and in cereal grains and oilseed meals such as soybean meal (**SBM**), phytate may account for 60 to 90% of total P (Ravindran et al., 1994). For phytate-bound P to become available to pigs, the IP_6 molecule must be hydrolyzed by the enzyme phytase, which sequentially removes phosphate groups and releases inorganic P (Wodzinski and Ullah, 1996; Greiner and Konietzny, 2006). However, pigs have limited endogenous phytase activity, resulting in low hydrolysis of phytate and poor utilization of phytate-bound P (She et al., 2018), and a substantial proportion of phytate bound P is excreted in the manure (Simons et al., 1990; Jongbloed and Lenis, 1998). Furthermore, phytate is a highly negatively charged molecule that readily chelates divalent and trivalent cations, particularly Ca, Mg, Zn, and Fe, forming insoluble phytate–mineral complexes in the gastrointestinal tract (Graf, 1986; Cosgrove, 1980; Reddy et al., 1982; Selle et al., 2000). Calcium has a strong affinity for phytate, and increasing dietary Ca concentration and Ca:P ratio can markedly reduce mineral solubility and availability (Champagne, 1996). In addition to mineral binding, phytate can interact with proteins and starch, either directly or indirectly,

reducing enzymatic access and digestion of amino acids (**AA**) and energy-yielding nutrients (Graf, 1986; Wodzinski and Ullah, 1996; Selle et al., 2012). Therefore, chelating properties of phytate contribute to decreased apparent and standardized digestibility of P, Ca, and trace minerals, and also impair AA and energy utilization (Graf, 1986; Selle et al., 2000). These anti-nutritional effects provide the physiological and economic basis for the development of microbial phytase as a commercial ingredient in diets for pigs and poultry.

Phytase and phytate hydrolysis: Overview

Early descriptions of the P-rich seed inclusions associated with "phytin" are commonly attributed to Hartig in 1855 and isolated by Pfeffer in 1872 (Crosgrove, 1980). Later work established that phytin is mainly a mixed salt form of phytate. Chemically, phytate is myo-inositol IP_6. In seeds, it accumulates during seed development as a major storage form of P for germination and early growth (Lott et al., 2000). Phytate is negatively charged, allowing interactions with minerals in feed and in the gut. For this reason, phytic acid was described as an antinutritional factor in the 1940s because phytate reduces mineral utilization (McCance and Widdowson, 1942; Cheryan, 1980; Selle and Ravindran, 2008). Phytate may form binary complexes with proteins (especially at low pH) and can also participate in ternary complexes (for example, protein-mineral-phytate) when minerals are present and pH is higher (Cheryan, 1980; Selle et al., 2009). However, treating SBM with a mold phytase increased the availability of phytate phosphorus, which indicated that phytate bound P can be released by phytase (Nelson et al., 1968; Nelson et al., 1971). Phytases catalyze sequential dephosphorylation of IP_6, releasing inorganic phosphate and producing lower inositol phosphates (IP_5, IP_4, IP_3, etc.), and finally free myo-inositol when degradation proceeds far enough (Schlemmer et al., 2001; Rosenfelder-Kuon et al., 2020).

The first commercial phytase products were launched in 1991 (Greiner and Konietzny, 2006; Selle and Ravindran, 2008), which was at a time when it was recognized that P output from intensive livestock systems needed to be reduced (Simons et al., 1990; Selle and Ravindran, 2008). One challenge in evaluating phytase-mediated hydrolysis of phytate is that the response depends on dietary conditions and the gut environment, particularly the dietary level of Ca (Selle and Ravindran, 2008; Dersjant-Li et al., 2015). Elevated levels of dietary Ca decreases P digestibility (Stein et al., 2011), because Ca promotes the formation of insoluble Ca–phytate complexes, which reduces phytate solubility and limits its accessibility to phytase as digesta pH increases along the gastrointestinal tract, and therefore, the level of dietary Ca and the Ca: digestible P ratio are important for responses to microbial phytase (Selle and Ravindran, 2008). Therefore, with high concentration of microbial phytase, IP_6 disappearance at the end of the ileum can be significant although pre-cecal P digestibility may not always increase in the same proportion as IP_6 degradation (Rosenfelder-Kuon et al., 2020; Lagos et al., 2023).

Effects of phytase on amino acids and energy digestibility

Beyond P utilization, phytase may also have "extra-phosphoric" effects, which may result in increased AA and energy digestibility. Phytate may form binary complexes with proteins and ternary complexes (protein-mineral-phytate), which may reduce protein solubility and enzyme access, but microbial phytase may mitigate these interactions by hydrolyzing the ester bonds that bind P to the inositol ring in phytate (Cheryan, 1980; Dersjant-Li et al., 2015). However, AA and energy responses to microbial phytase are not consistent. In some cases, phytase increased phytate degradation without improving ileal AA digestibility (She et al., 2018; Mesina et al., 2019; Rosenfelder-Kuon et al., 2020) whereas, phytase improved apparent ileal AA digestibility and/or improved apparent total tract digestibility of energy and nutrients in other experiments

(Liao et al., 2005; Espinosa et al., 2022; Lagos et al., 2023). This inconsistency reflects several interacting factors: phytate chemistry and solubility, dietary Ca concentration and Ca: digestible P ratio, ingredient matrix complexity, phytase source and dose, and how much phytate is degraded before the terminal ileum (Selle and Ravindran, 2008; Dersjant-Li et al., 2015).

Potential interaction with phytase and vitamin D metabolites

Vitamin D and its metabolites regulate Ca and P homeostasis through endocrine control of intestinal absorption, renal handling, and bone remodeling (Jones et al., 1998; Combs and McClung, 2017). However, because phytase increases luminal release of Ca and P from phytate complexes, an interaction between phytase and vitamin D metabolites is possible because the action of microbial phytase is different from that of vitamin D (Selle and Ravindran, 2008; Selle et al., 2009; Dersjant-Li et al., 2015). However, there is a lack of data demonstrating combined effects of phytase and vitamin D metabolite when included in diets for pigs. Results of a recent experiment with growing pigs indicated that microbial phytase and 25-hydroxyvitamin D_3 increased digestibility of Ca and P and influenced plasma vitamin D metabolites and serum bone biomarkers, but effects were not always additive (Jaramillo et al., 2025). Therefore, the interaction between improved luminal mineral release (from phytate hydrolysis) and vitamin D mediated absorption of Ca and P is not incompletely understood. In sows, supplementation with 25(OH)D_3 improved Ca and P balance and increased ATTD of DM and GE as well as DE and ME, especially in phytase-free diets, whereas microbial phytase increased Ca and P balance but did not increase DE or ME (Lee et al., 2022).

Soybean meal in pig nutrition: amino acids, energy, and phytate bound-P

Soybean meal is the most widely used protein ingredient in pig diets and in corn-based

formulations, SBM is the main source of indispensable AA. Corn contributes to dietary energy but has a relatively low concentration of Lys and other indispensable AA (NRC, 2012). For this reason, SBM supplies most of the digestible AA (especially Lys) needed to meet requirements by growing pigs (Sotak-Peper et al., 2015; Lagos and Stein, 2017). A practical advantage of SBM is that its AA profile complements cereal grains and, when properly processed, SBM provides high standardized ileal digestibility (**SID**) of AA. However, the nutritive value of SBM is not constant (Lagos and Stein, 2017). Differences in soybean origin and processing conditions may change nutrient concentrations and SID of AA, which makes it important to use digestibility-based values rather than total AA concentrations when formulating diets (Lagos and Stein, 2017). In addition, SBM contains antinutritional factors (e.g., trypsin inhibitors and oligosaccharides) and these factors can influence nutrient utilization, particularly in young pigs or when processing is suboptimal (Oliveira and Stein, 2016).

In addition to AA, SBM also contributes substantial energy to diets, and on average, SBM produced in the United States contain 4,044 kcal metabolizable energy/kg dry matter (Sotak-Peper et al., 2015). In several experiments it was demonstrated that metabolizable energy of SBM is greater than in corn (Rojas et al., 2013). Therefore, when SBM inclusion changes not only AA digestibility but also energy digestibility and energy contribution, need to be considered.

Phytate and phosphorus in soybean meal

In corn–SBM diets, SBM contains more phytate (and phytate-bound P) than corn. As an example phytate concentration in SBM is 1.60% vs. 0.63% in corn (as-fed basis), and phytate-bound P is 0.45% in SBM vs. 0.18% in corn (She et al., 2018; Lee et al., 2023). Thus, phytate-bound P

represents a large proportion of total P in both ingredients, but SBM has a greater absolute amount of phytate and phytate bound-P (She et al., 2018). This difference is important in practical formulation because SBM is included at levels that are sufficient to supply digestible AA, and that inclusion level often contributes a large share of the total dietary phytate substrate (Ravindran et al., 1994). Although SBM always is included at a lower percentage than corn, the greater phytate concentration in SBM can make SBM a major contributor to total dietary phytate (She et al., 2018). As a result, when phytase is added to corn–SBM diets, a large part of the released P can originate from phytate hydrolysis associated with SBM, and not only from the grain fraction (Sotak-Peper et al., 2016; She et al., 2018). However, phytate degradation is also affected by dietary Ca concentration and gastrointestinal pH, and therefore the amount of P that is released by the action of phytase is not determined only by the concentration of phytate in the diet, but also by conditions that influence solubility and enzyme access (Champagne, 1996; Selle et al., 2000). Increasing dietary Ca can reduce P digestibility in pigs, which is a result of formation of less soluble Ca–phytate complexes and reduced effective phytate hydrolysis/availability under some conditions (Stein et al., 2011). Nevertheless, in soybean ingredients, phytase consistently increases P digestibility, and this has been demonstrated for both conventional and fermented SBM (Rojas and Stein, 2012) and for SBM produced in different areas of the United States (Sotak-Peper et al., 2017). Likewise, increasing supplemental phytase gradually improves Ca and P digestibility, whereas increased AA digestibility is not consistently observed for all AA (Traylor et al., 2001; She et al., 2018). However, most experiments only up to 3,000 units of microbial phytase were used and there is a lack of information about effects of using greater levels of phytase in diets containing SBM and fed to growing pigs

Conclusions

Phytate is the main storage form of P in plant feed ingredients, pigs have limited endogenous phytase activity, and therefore digestibility of phytate-bound P is low. Because of that, inorganic phosphate sources are often added to diets for pigs which increases feed cost. However, inclusion of microbial phytase in the diets releases phytate bound P, which reduces the need for adding feed phosphates to the diets. In addition, the use of microbial phytase improves not only P digestibility, but also Ca digestibility and reduces P excretion. The efficacy of the phytase response depends on factors such as Ca levels in diets and the Ca: digestible P ratio, and the phytase dose. Phytase may also improve amino acid and energy utilization by reducing anti-nutritional effects of phytate chelation of AA and starch, but data to confirm this hypothesis are needed. Finally, the use of vitamin D_3 metabolites such as 25(OH)D_3 can increase the digestibility of Ca and P, but some bone biomarkers related to bone synthesis have not been shown to increase in response to this metabolite. However, vitamin D_3 and phytase increased concentrations of osteocalcin, which is not only an osteoblast-derived protein but also acts as a regulator of insulin, and may therefore be related to energy metabolism and, to some extent, performance. More studies are needed to evaluate the additional effects of vitamin D_3 on Ca and P utilization in growing pigs.

Figure

Figure 2.1

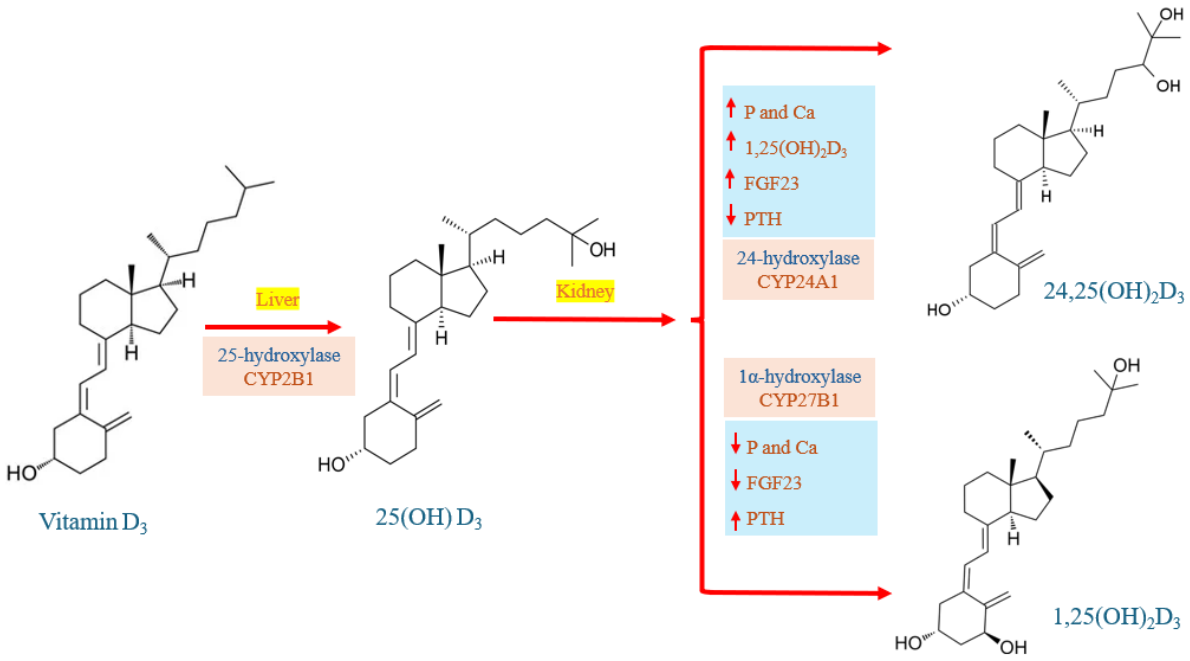

Figure 2.1 Metabolism of vitamin D_3.

Adapted from, Combs & McClung 2017

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Chapter 3: Microbial phytase and 25-hydroxy-vitamin D3 fed to growing pigs increase digestibility of calcium and phosphorus and influence plasma vitamin D metabolites and serum bone biomarkers, but effects are not always additive[1]

Abstract

The objective was to test the hypothesis that calcifediol [**25(OH)D_3**] and microbial phytase have additive effects on the standardized total tract digestibility (**STTD**) of Ca and P, serum bone biomarkers, and plasma vitamin D_3 metabolites when fed to growing pigs. Sixty barrows (initial body weight: 25.98 ± 2.01 kg) were housed individually in metabolism crates and assigned to a randomized complete block design with three blocks, 5 diets, and 12 replicate pigs per diet. The positive control (**PC**) diet was formulated to meet Ca and P requirements of growing pigs. Four additional diets contained 75% of the required Ca and P and were used in a 2 × 2 factorial design with 0 or 50 μg/kg of 25(OH)D_3 and 0 or 500 units of phytase per kg diet. Pigs were fed experimental diets for 13 days that included a 5-day adaptation period and a 5-day fecal collection period. Fecal samples were analyzed for Ca and P and the STTD of Ca and P were calculated. Blood samples were collected on days 1 and 13 to measure bone alkaline phosphatase, osteocalcin, type 1 collagen, and fibroblast growth factor 23. Analyzed plasma vitamin D_3 metabolites included 25(OH)D_3, 24,25 dihydroxycholecalciferol [**24,25(OH)$_2$D$_3$**], and 1,25 calcitriol [**1,25(OH)$_2$D$_3$**]. Results indicated that the STTD of P was greater ($P < 0.05$) in

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the PC diet than in the diet containing 75% of the required Ca and P and no microbial phytase or 25(OH)D_3. The STTD of Ca and P increased ($P < 0.001$) in pigs fed diets containing phytase, and STTD of Ca and P tended to increase if 25(OH)D_3 was added to the diet, but only in the absence of phytase (interaction; $P < 0.10$). On day 13, osteocalcin, which is a biomarker for bone tissue synthesis, was increased ($P < 0.05$) if 25(OH)D_3 or phytase was added to diets, but the other bone biomarkers did not differ among treatments. Plasma 25(OH)D_3 and 24,25(OH)$_2D_3$ increased ($P < 0.05$) if diets contained 25(OH)D_3 and(or) phytase, indicating increased metabolic activity of vitamin D_3. Plasma 1,25(OH)$_2D_3$ was greater ($P < 0.001$) in pigs fed the diet with 75% of the required Ca and P and no phytase or 25(OH)D_3 than in pigs fed the PC diet, but microbial phytase decreased ($P < 0.001$) plasma 1,25(OH)$_2D_3$. In conclusion, microbial phytase and 25(OH)D_3 increased Ca and P digestibility and serum osteocalcin, and vitamin D_3 status was improved with addition of 25(OH)D_3 to the diet fed to growing pigs, but effects of 25(OH)D_3 and microbial phytase were not always additive.

Key words: 25(OH)D_3, calcium, phytase, phosphorus, pig, vitamin D

Abbreviations: ATTD, apparent total tract digestibility; BAP, bone alkaline phosphatase; CTX-I, carboxyterminal cross-linked telopeptide of type I collagen; DM, dry matter; FGF23, fibroblast growth factor; FTU, phytase units; NC, negative control; OC, osteocalcin; PC, positive control; STTD, standardized total tract digestibility; VMR, vitamin D metabolites ratio; 25(OH)D_3, 25 calcifediol; 24,25(OH)$_2D_3$, 24,25 dihydroxycholecalciferol; 1,25(OH)$_2D_3$, 1,25 calcitriol.

Introduction

Vitamin D is important in bone development, prevention of tetany, and in Ca and P homeostasis, and has other physiological functions related to growth, maintenance, and health

(DeLuca, 2004). Cholecalciferol (i.e., vitamin D_3) is the main source of vitamin D in animal diets (Stein, 2024), but vitamin D_3 is inactive and must be hydroxylated to 25-hydroxycholecalciferol [**25(OH)D_3**] in the liver, and then a second hydroxylation occurs in the kidney to produce 1,25 dihydroxycholecalciferol [**1,25(OH)$_2$$D_3$**], which is the active form of vitamin D_3 (Combs and McClung, 2017). Supplementation of 25(OH)D_3 to diets for sows in late gestation increased digestibility of P and Ca, increased bone mineralization, and improved blood vitamin D_3 status (Lauridsen et al., 2010). In laying hens, long-term supplementation of 25(OH)D_3 resulted in improved bone growth, increased bone volume, and improved bone quality (Chen et al., 2020).

In diets for pigs based on cereal-grains and oilseed meals, most P is unavailable because it is bound to phytate, resulting in low P digestibility in grain-based diets (Liao et al., 2005). However, use of exogenous microbial phytase in diets for pigs results in hydrolysis of the ester bonds between P and phytate, which results in increased digestibility of Ca and P by pigs with a subsequent increase in absorption of P (Pallauf et al., 1994; Almeida et al., 2013). Inclusion of 25(OH)D_3 in diets for gestating sows increased the apparent total tract digestibility (**ATTD**) and retention of Ca and P and it appeared that the increase in digestibility of Ca and P caused by 25(OH)D_3 was independent of the increase in digestibility caused by phytase (Lee et al., 2022). These results were obtained in diets containing vitamin D_3 well above the requirement for gestating sows and it was, therefore, concluded that sows may not be efficient in converting vitamin D_3 to 1,25(OH)$_2$$D_3$ (Lee et al., 2022). However, it is not known if growing pigs also have difficulty converting vitamin D_3 to 1,25(OH)$_2$$D_3$, but if that is the case, it is expected that addition of 25(OH)D_3 to the diets will have a positive effect on digestibility of Ca and P. There is also limited information about a possible additive effect of 25(OH)D_3 and microbial phytase in diets fed to growing pigs. Therefore, the hypothesis for this experiment was that effects of

25(OH)D_3 and microbial phytase on ATTD and standardized total tract digestibility (**STTD**) of Ca and P in diets fed to growing pigs are additive and that both 25(OH)D_3 and microbial phytase will result in increased serum biomarkers for bone synthesis and resorption and optimal vitamin D_3 metabolite status in plasma.

Materials and methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment before the animal work was initiated. Pigs used in the experiment were the offspring of L800 males mated to Camborough sows (PIC, Hendersonville, TN, USA).

Animals, housing, diets, feeding, and sample collection

Sixty growing male pigs with a body weight of 25.98 ± 2.01 kg were allotted to one of five diets in 3 blocks with weaning group as the blocking factor. Each block had 20 pigs (4 pigs per diet) for a total of 12 replicate pigs per treatment. Pigs were housed in metabolism crates (0.81 m × 1.52 m) that were equipped with a self-feeder, a nipple drinker, and a slatted floor.

Five corn-soybean meal-based diets were formulated (Table 3.1). The positive control (**PC**) diet contained total Ca and digestible P at the recommended levels for 25 to 50 kg growing pigs (NRC, 2012). A negative control (**NC**) diet was formulated with 75% of the requirement of Ca and digestible P and this diet, therefore, contained 0.17 percentage units total Ca and 0.08 percentage units STTD P less than the PC diet. Three additional diets were formulated by supplementing NC with either 50 µg/kg of 25(OH)D_3 (Hy-D®; DSM, Parsippany, NJ, USA), 500 phytase units (**FTU)** per kg (HiPhorius®; DSM, Parsippany, NJ, USA), or both 50 µg/kg 25(OH)D_3 and 500 FTU/kg. The Ca to digestible P ratio was maintained at 2.13:1 in all diets. All vitamins and minerals other than Ca and P were included in all diets to meet or exceed current

nutrient requirements (NRC, 2012). The daily feed allowance was calculated as 3.2 times the maintenance requirement for metabolizable energy based on the initial body weight of pigs (i.e., 197 kcal metabolizable energy/kg body weight$^{0.60}$; NRC, 2012). Feed allotments were provided in two daily meals that were fed at 0730 and 1530 h. Water was available at all times.

Experimental diets were fed for 13 days. The initial 5 days were considered the adaptation period to the diets and the adaptation period was followed by 5 days of fecal collection using the marker-to-marker procedure (Adeola, 2001). The start marker was fed in the morning meal on day 6 and the stop marker was fed in the morning meal on day 11. Fecal collection was initiated when the first marker (i.e., indigo carmine) appeared in the feces and ceased when the second marker (i.e., ferric oxide) appeared (Adeola, 2001). Fecal samples were stored at –20 °C as soon as collected, and at the conclusion of the experiment, samples were dried at 65 °C in a forced air oven (Heratherm OMH750; Thermo Fisher 1873 Scientific Inc., Waltham, MA, USA) and finely ground through a 0.5-mm screen using a hammermill (model: MM4; Schutte, Buffalo, NY, USA).

On days 1 and 13, one blood sample was collected via venipuncture three hours after the morning meal to determine bone biomarker concentrations. Samples were collected in a 10-mL red-top vacutainer, which did not contain any coagulant. The blood in the tube was allowed to clot and serum was then harvested. Additionally, on day 1, two pigs per diet were randomly selected, and a blood sample was collected in a 10-mL purple-top vacutainer containing the anticoagulant ethylenediaminetetraacetic acid (**EDTA**). After 13 days on experimental diets, a second sample was collected from five randomly selected pigs per diet (25 in total). Blood samples were immediately centrifuged, and plasma and serum were harvested and stored at –20 ºC. Samples were stored at –80 °C until analysis.

Chemical analysis

Before the animal part of the experiment was initiated, the concentration of 25(OH)D_3 in diets was analyzed at Technical Marketing Analytical Service (dsm-firmenich; Belvidere, NJ, USA) using liquid chromatography with tandem mass spectrometry (Aronov et al., 2008; Table 3.2). Concentrations of Ca and P in feed ingredients, diets, and dried fecal samples were analyzed (method 985.01 A, B and C; AOAC Int., 2019) using inductively coupled plasma-optical emission spectrometry (Avio 200, PerkinElmer, Waltham, MA, USA; Table 3.2). Sample preparation included dry ashing at 600 °C for 4 h according to method 942.05 (AOAC Int., 2019) and wet digestion with nitric acids according to method 3050 B (U.S.-EPA, 2000). Corn and soybean meal were analyzed for phytate (Ellis et al., 1977) and phytate in diets was calculated based on the analyzed phytate in corn and soybean meal and the inclusion rates of corn and soybean meal in each diet. Phytase activity in diets was also analyzed according to method 2000.12 (AOAC Int., 2019). Corn, soybean meal, diets, and fecal samples were analyzed for dry matter (**DM;** method 930.15; AOAC Int., 2019) and ash (method 942.05; AOAC Int., 2019). Limestone and dicalcium phosphate were analyzed for ash as well. Diets, corn, and soybean meal were also analyzed for gross energy using bomb calorimetry (Model 6400, Parr Instruments, Moline, IL, USA) and for insoluble dietary fiber and soluble dietary fiber using the Ankom Dietary Fiber Analyzer (Ankom Technology, Macedon, NY; method 991.43, AOAC Int., 2019). Total dietary fiber was calculated as the sum of soluble and insoluble dietary fiber. Crude protein was calculated as analyzed nitrogen × 6.25 and nitrogen in diets, corn, and soybean meal were analyzed by combustion (method 990.03; AOAC Int., 2019) using a LECO FP628 Nitrogen Analyzer (LECO Corp., Saint Joseph, MI, USA). Serum osteocalcin (**OC**) was

determined using an N-MID® Osteocalcin Enzyme-Linked Immunosorbent Assay Kit (Immunodiagnostic Systems Ltd, The Boldons, UK). Serum carboxy-terminal cross-linked telopeptide of type I collagen (**CTX-I**) was determined using a Pig Cross-Linked C-Telopeptide of Type I Collagen Enzyme-Linked Immunosorbent Assay Kit (Abbexa Ltd., Cambridge, UK). Serum bone-specific alkaline phosphatase (**BAP**) was determined using Ostase® BAP Enzyme Immunoassay Kit (Immunodiagnostic Systems Ltd, The Boldons, UK). Serum fibroblast growth factor (**FGF23**) concentration was determined using My BioSource® FGF23 competitive enzyme immunoassay Kit (Biotech, Life Sciences, Manufacturing, San Diego, USA). Plasma 1,25$OH)_2D_3$, 24,25 dihydroxycholecalciferol [**24,25$(OH)_2D_3$**], and 25$(OH)D_3$ were determined using liquid chromatography-mass spectrometry (LC/MS/MS; AOAC., 2019) by Heartland Assays (Ames, IA, USA).

Calculations and statistical analysis

Concentrations of phytate-bound P were calculated by multiplying the analyzed phytate by 0.282 (Tran and Sauvant, 2004) and non-phytate P was calculated by subtracting phytate-bound P from total P. Feed intake was calculated by subtracting the weight of dried orts from feed provisions. The ATTD of Ca and P in diets was calculated (Almeida and Stein, 2010) and the STTD of P was calculated by correcting ATTD for the basal endogenous loss of P (i.e., 190 mg/kg DM intake; NRC, 2012). The ratio between 24,25$(OH)_2D_3$ and 25$(OH)D_3$, known as the vitamin D metabolite ratio (**VMR**), was used to assess optimal functional vitamin D_3 status (Cavelier et al., 2020).

Normality and homogeneity of data were verified using the UNIVARIATE and MIXED procedures (SAS Inst. Inc., Cary, NC, USA). Outliers were detected using Internally Studentized Residuals (Tukey, 1977). Pig was the experimental unit for all analyses. Data were analyzed

using MIXED procedures of SAS. The statistical model included diet as fixed effect and block and replicate pig within block as random effects. For analyzing data for serum biomarkers on day 13, concentrations of the biomarkers on day 1 were used as a covariate in the model. Plasma vitamin D_3 metabolites analyzed in samples from day 1 were assumed to be representative for all pigs and changes in plasma vitamin D_3 metabolites from day 1 to 13 were analyzed. Least squares means were calculated using the LSMeans statement in SAS. Contrast coefficients were used to determine effects of 25(OH)D_3, phytase, and the interaction between 25(OH)D_3 and phytase when Ca and P were low. Differences between PC and NC diets containing no 25(OH)D_3 or phytase were also analyzed using a contrast statement. Statistical significance and trends were considered at $P < 0.05$ and $P < 0.10$, respectively.

Results

Pigs remained healthy during the experiment and limited feed refusals were observed. Diet analysis confirmed correct mixing.

Standardized total tract digestibility of Ca

Results indicated that feed intake was not different between pigs fed the PC and NC diets (Table 3.3). Fecal excretion, Ca intake, Ca excretion in feces, and absorbed Ca were greater ($P < 0.05$) for the PC diet than the NC diet, but the ATTD of DM was greater ($P < 0.05$) in the NC diet than in the PC diet. However, there were no differences in the ATTD of Ca, the basal endogenous loss of Ca, or the STTD of Ca between PC and NC.

Supplementation of 25(OH)D_3 to the NC diet did not affect feed intake, fecal excretion, or the ATTD of DM in pigs. Calcium intake and absorbed Ca increased ($P < 0.01$) if 25(OH)D_3 was added to the NC diet, but Ca excretion in feces was not affected by supplemental 25(OH)D_3.

Feed intake, fecal excretion, and the ATTD of DM were not affected by supplemental phytase. Calcium intake and Ca excretion in feces were reduced ($P < 0.01$) if phytase was included in the diet, whereas absorbed Ca, the ATTD of Ca, and the STTD of Ca increased ($P < 0.001$) if phytase was used. Both 25(OH)D_3 and phytase increased the ATTD and STTD of Ca, but the increase caused by 25(OH)D_3 was less if phytase was used than if no phytase was used (tendency for interaction; $P = 0.064$, and $P = 0.072$, respectively).

Standardized total tract digestibility of P

Phosphorus intake was less ($P < 0.001$) for pigs fed the NC diet than for pigs fed the PC diet. Phosphorus excretion in feces and the basal endogenous loss of P were not different between PC and NC diets, but absorbed P, the ATTD of P, and the STTD of P were less ($P < 0.05$) for pigs fed the NC diet than the PC diet.

Phosphorus intake increased ($P < 0.001$) if 25(OH)D_3 was added to the diet, whereas phytase tended ($P = 0.083$) to reduce P intake. Excretion of P was reduced if 25(OH)D_3 was added to the diet without phytase, but that was not the case if the diet also contained phytase (interaction, $P < 0.01$). However, phytase reduced ($P < 0.001$) P excretion in feces regardless of the level of 25(OH)D_3 in the diet. Likewise, phytase increased ($P < 0.001$) absorbed P, ATTD of P, and STTD of P both in the diet without and with 25(OH)D_3,whereas 25(OH)D_3 increased absorption of P, ATTD of P, and STTD of P in diets that did not contain phytase, but not in diets with phytase (interaction, $P < 0.01$).

Bone biomarkers

On day 13, there were no differences in serum levels of OC, BAP, CTX-I, OC to CTX-I ratio, or FGF23 between PC and NC diets (Table 3.4). Serum OC was increased ($P < 0.01$) by dietary 25(OH)D_3, but BAP, CTX-I, OC to CTX-I ratio, or FGF23 were not influenced by supplemental

25(OH)D_3. Regardless of 25(OH)D_3, supplemental phytase increased ($P < 0.001$) serum OC, but serum levels of BAP, CTX-I, OC to CTX-I ratio, or FGF23 were not affected by phytase.

Blood vitamin D biomarkers

On day 13, pigs fed PC had greater ($P < 0.05$) plasma 24,25(OH)$_2$$D_3$ and VMR than pigs fed NC, whereas plasma concentration of 1,25(OH)$_2$$D_3$ was greater ($P < 0.001$) in pigs fed NC than in pigs fed PC (Table 3.5). Addition of 25(OH)D_3 or microbial phytase to the diet increased ($P < 0.01$) plasma concentrations of 25(OH)D_3 and 24,25(OH)$_2$$D_3$, whereas 1,25(OH)$_2$$D_3$ in plasma was reduced ($P < 0.001$) if phytase was added to the diet. The VMR increased if 25(OH)D_3 was added to the diet without phytase, but that was not the case if phytase was included in the diet (interaction, $P < 0.05$). However, Phytase increased ($P < 0.01$) VMR regardless of the level of 25(OH)D_3 in the diet.

Changes from day 1 to 13 in plasma 25(OH)D_3 and 24,25(OH)$_2$$D_3$ were not affected by dietary Ca and P, but compared with PC, plasma VMR was reduced ($P < 0.01$) and 1,25(OH)$_2$$D_3$ increased ($P < 0.01$) from day 1 to 13 in pigs fed the NC diet (Table 3.6). Supplementing 25(OH)D_3 to the diet increased ($P < 0.001$) plasma 25(OH)D_3 and 24,25(OH)$_2$$D_3$ from day 1 to 13, but the change in plasma 1,25(OH)$_2$$D_3$ from day 1 to 13 was not affected by supplemental 25(OH)D_3. Adding 25(OH)D_3 to the diet also increased VMR from day 1 to day 13, but more so in diets without phytase than in diets with phytase (tendency for interaction, $P = 0.057$). Supplementation of phytase did not change plasma 25(OH)D_3, 24,25(OH)$_2$$D_3$, or VMR, but reduced ($P < 0.01$) plasma 1,25(OH)$_2$$D_3$ on day 13 compared with day 1.

Discussion

Calcium and phosphorus digestibility

Analyzed concentrations of ash, Ca, and P in corn, soybean meal, limestone, and dicalcium phosphate were in agreement with published values (NRC, 2012) and analyzed diet concentrations of crude protein, gross energy, total dietary fiber, Ca, and P agreed with calculated values. Analyzed $25(OH)D_3$ in the two diets that contained this metabolite were 43 µg/g and 46 µg/g respectively, which corresponded to 86% and 92% of the expected value. Analyzed phytase was 424 and 426 FTU per kg in diets with phytase, which was 85% of the expected concentration.

The increase in ATTD and STTD of Ca and P when microbial phytase was added to diets agrees with results of previous experiments (Veum and Ellersieck, 2008., Almeida et al., 2013; Blavi et al., 2019), and the responses are within the range of results obtained for corn-soybean meal diets (Arredondo et al., 2019; Gebhardt et al., 2021). These results confirmed the efficiency of the phytase used in the experiment and are a result of the increased release of Ca and P from the phytate complex when phytase is added to the diet. The tendency for an increase in STTD of Ca in diets containing $25(OH)D_3$ is likely a result of the increased concentration of $1,25(OH)_2D_3$ in plasma of pigs fed $25(OH)D_3$, which may have increased transcellular absorption of Ca from the small intestine (Lagos et al., 2019). Likewise, the increase in absorbed P and the tendency for increased STTD of P that was observed when $25(OH)D_3$ was added to the diet is likely a result of the increased plasma concentration of $1,25(OH)_2D_3$ (Katai et al., 1999; Stein, 2024), because increased $1,25(OH)_2D_3$ results in increased expression of Ca channel proteins and Ca binding proteins in the enterocytes (González-Vega et al., 2016, Lagos et al., 2019). It therefore appears that although all diets contained vitamin D_3 in quantities that were believed to be well above the requirement for growing pigs (NRC, 2012), provision of the $25(OH)D_3$ metabolite in addition to the dietary vitamin D_3 was efficient in increasing plasma $1.25(OH)_2D_3$ and thus increase

absorption of Ca and P. This observation is in agreement with results from a recent experiment with gestating sows (Lee et al., 2022) and indicates that growing pigs are not able to fully convert dietary vitamin D_3 to $1.25(OH)_2D_3$. Theoretically, it is also possible that the requirement for vitamin D_3 is much greater than previously thought, which might have been the reason why addition of $25(OH)D_3$ to the diets tended to increase STTD of Ca and P if no phytase was used. However, because the inclusion of vitamin D_3 to diets used in this experiment was more than eight times greater than the requirement (NRC, 2012), it is unlikely that the added vitamin D_3 was below the requirement and it is, therefore, more likely that pigs are not able to efficiently convert dietary vitamin D_3 to $1.25(OH)_2D_3$.

An additive effect on Ca and P digestibility of using both $25(OH)D_3$ and phytase in diets fed to sows was observed by Lee et al. (2022), but such an effect was not observed in the current experiment. This observation is in agreement with data from growing-finishing pigs fed low P diets containing $25(OH)D_3$ or microbial phytase (O'Doherty et al., 2010). It therefore appears that for growing pigs, $25(OH)D_3$ only increases STTD of Ca and P if no phytase is used in the diets, whereas the effects are different in sows. It is possible that this is a result of sows being less efficient than growing pigs in converting vitamin D_3 into $1,25(OH)_2D_3$ (Stein, 2024). Nevertheless, the hypothesis that effects of $25(OH)D_3$ and phytase on STTD of Ca and P are additive was rejected. It is speculated that the reason for the lack of additivity between microbial phytase and $25(OH)D_3$ may be that when phytase was used, the availability of Ca and P was sufficient to meet the requirements of the pigs, which then resulted in down regulation of the transcellular absorption of Ca and P, and thus resulting in no measurable effect of $25(OH)D_3$ on Ca and P digestibility. In contrast when no microbial phytase was used, expression of Ca channel proteins and intracellular transport proteins was stimulated by $25(OH)D_3$, which resulted in

increased absorption of Ca and P. If this hypothesis is correct, it is possible that the NC diet needs to be more deficient in Ca and P to demonstrate additive effects of microbial phytase and 25(OH)D_3.

Bone turnover biomarkers and FGF23

Bone turnover biomarkers in serum were analyzed to provide an assessment of the bone tissue status in pigs, as they may reflect changes in bone integrity, especially when dietary Ca and P levels are changed (Liesegang et al., 2002). Greater absorption of Ca and P stimulates osteoblast activity and increase bone tissue formation and serum OC levels (Sørensen et al., 2018). Thus, the increase in OC that was observed when 25(OH)D_3 or phytase was added to the diet, indicates that osteoblast activity, and therefore bone tissue synthesis, was stimulated by dietary 25(OH)D_3 and microbial phytase. Low or high concentrations of Ca and P in diets for weanling or growing-finishing pigs are believed to increase BAP, and in low Ca and P diets, CTX-I increases as well (Liesegang et al., 2002; Sørensen et al., 2018; Lee et al., 2020). Therefore, the observation that BAP and CTX-I did not increase when diet Ca and P were reduced is in contrast with results of previous experiments (Lee et al., 2020). It is possible that the length of the experiment was too short to detect changes in BAP and CTX-I, as it may take more than 13 days for these biomarkers to exhibit measurable changes in serum (Carter et al., 1996; Liesegang et al., 2002; Sørensen et al., 2018). Thus, the hypothesis that dietary 25(OH)D_3 and/(or) phytase increase biomarkers for bone tissue synthesis and reduce biomarkers for bone resorption was only partially accepted because only OC was increased by dietary treatments.

Plasma FGF23 is a hormone involved in negative feedback with 1,25$(OH)_2D_3$ to downregulate reabsorption of phosphate in the kidneys, which increases P excretion in urine and decreases P retention (Agoro and White, 2023; Vötterl et al., 2023). Therefore, it was expected

that plasma FGF23 would be greater in pigs fed the PC diet than in pigs fed the NC diet, and that FGF23 would be increased when 25(OH)D_3, phytase, or both 25(OH)D_3 and phytase were supplemented to the NC diet because of increased Ca and P availability in the body (Hasan et al., 2022). However, the observation that plasma FGF23 was not affected by dietary Ca and P or by supplementation of 25(OH)D_3 or phytase was in agreement with previous data (Oster et al., 2016), and it is possible that because FGF23 is a hormone, the concentration is tightly regulated, which is the reason for the lack of impact of diet on plasma FGF23. However, further research is needed to understand the regulatory mechanisms of plasma FGF23 in response to dietary treatments in pigs.

Vitamin D_3 plasma metabolites

The vitamin D_3 metabolites were analyzed in plasma to determine effects of dietary Ca and P concentration and addition of 25(OH)D_3 or phytase on concentration of 1,25$(OH)_2D_3$. The observation that plasma 25(OH)D_3 on day 13 was greater in pigs fed diets containing 25(OH)D_3 than in pigs fed no 25(OH)D_3 indicates that more 25(OH)D_3 was available for conversion to 1,25$(OH)_2D_3$ (Cavelier et al., 2020; Upadhaya et al., 2021), which may explain the increased absorption of Ca and P by pigs fed 25(OH)D_3 although no significant increase in plasma 1,25$(OH)_2D_3$ was caused by 25(OH)D_3. This observation is in agreement with data from weanling pigs, which also indicated that serum 25(OH)D_3 increased when pigs were fed a diet containing 25(OH)D_3 (Becker et al., 2024). However, it is acknowledged that plasma concentrations of the metabolites, which were measured in this experiment, may not always be representative of metabolic flux and substrate conversion due to the big difference in half-life between 25(OH)D_3 and 1.25$(OH)_2D_3$.

The observation that inclusion of either 25(OH)D_3 or phytase in the diets resulted in increased plasma 25(OH)D_3 and 24,25(OH)$_2D_3$ is in agreement with the increases in serum OC that were observed and supports the hypothesis that both metabolites may be needed for bone plate growth and mineralization (Boyan et al., 2001; Zelzer et al., 2020, Becker et al., 2024). The observation that 1,25(OH)$_2D_3$ was reduced in plasma of pigs fed phytase indicates that the increased intestinal availability of Ca and P that was caused by phytase reduced the need for 1,25(OH)$_2D_3$ to aid in absorption of Ca and P (Cavelier et al., 2020; Dugar et al., 2023). Because 25(OH)D_3 increased VMR, it is likely that 25(OH)D_3 partly prevented the negative impact of low Ca and P in diets without microbial phytase. The greater level of plasma 1,25(OH)$_2D_3$ in pigs fed the NC than PC diets indicates that more vitamin D_3 was activated to 1,25(OH)$_2D_3$ in pigs fed the NC diet, which may have aided in increasing absorption of Ca and P to maintain homeostasis (DeLuca, 2004).

The observation that 1,25(OH)$_2D_3$ was reduced from day 1 to day 13 in PC indicates that if diets meet the requirements for Ca and P, the need for activating vitamin D receptors is reduced over time, which may be a result of the reduced requirement for Ca and P as pigs grow. The metabolite 24,25(OH)$_2D_3$ is generated after degradation of 1,25(OH)$_2D_3$ or 25(OH)D_3 to reduce the concentration of active vitamin D (Lida et al., 1995). The increase in plasma 24,25(OH)$_2D_3$ that was observed when diets were supplemented with 25(OH)D_3 or phytase was expected because pigs fed these diets had a reduced need for synthesizing 1,25(OH)$_2D_3$. It thus seems that 24,25(OH)$_2D_3$ is an indicator of vitamin D_3 catabolism and overall vitamin D_3 metabolic activity in pigs.

Limitations to this experiment include that for some of the blood analyses we had to reduce the replications due to the high cost of these analyses. It is also acknowledged that the

length of the experiment was short, and it is possible that some of the blood measurements would be different if diets were fed for a longer time. It is also possible that if bone strength had been measured using a DEXA scan, additional information about the impact of 25(OH)D_3 and phytase on bone formation might have been obtained.

Conclusion

Supplementation of diets for growing pigs with microbial phytase and/(or) 25(OH)D_3 resulted in increased STTD of Ca and P. However, 25(OH)D_3 only had a positive effect on STTD of Ca and P if no phytase was used, indicating that effects of 25(OH)D_3 and phytase are not additive in growing pigs. It is, however, possible that diets with lower concentrations of Ca and P than used in this experiment are needed to demonstrate additive effects between microbial phytase and 25(OH)D_3. The increased STTD of Ca and P caused by microbial phytase or 25(OH)D_3 were reflected in elevated serum OC levels, which indicated increased bone tissue synthesis. Inclusion of 25(OH)D_3 or phytase in the diets also improved plasma 24,25$(OH)_2D_3$, which indicates increased vitamin D_3 metabolism.

Disclosures

The authors have no conflicts of interest.

Tables

Table 3.1. Ingredient composition of experimental diets, as-fed basis

Item	Ca and P:	Normal[1]	75% of requirements			
		PC[2]	NC[2]	NC	NC	NC
	25(OH)D_3:	-	-	+	-	+
	Phytase:	-	-	-	+	+
Corn		70.89	71.55	71.05	71.05	70.55
Soybean meal		25.50	25.50	25.50	25.50	25.50
Soybean oil		0.40	0.40	0.40	0.40	0.40
Dicalcium phosphate		0.98	0.48	0.48	0.48	0.48
Calcium carbonate		0.84	0.69	0.69	0.69	0.69
$_{L}$-Lys·HCl, 78.8% Lys		0.30	0.30	0.30	0.30	0.30
$_{DL}$-Met, 99% Met		0.10	0.10	0.10	0.10	0.10
$_{L}$-Thr, 99% Thr		0.09	0.09	0.09	0.09	0.09
Sodium chloride		0.40	0.40	0.40	0.40	0.40
Corn-25(OH)D_3 premix[3]		-	-	0.50	-	0.50
Corn-phytase premix[4]		-	-	-	0.50	0.50
Vitamin-mineral premix[5]		0.50	0.50	0.50	0.50	0.50

[1]Normal = diets containing Ca and P at 100% of the requirement (NRC, 2012).

[2]PC = positive control; NC = negative control.

[3]The corn-25(OH)D_3 premix was prepared by mixing 362.84 g of 25(OH)D_3 concentrate (137.8 mg/kg; Hy-D®; DSM, Parsippany, NJ, USA) and 1,387.16 g ground corn. At 0.50 % inclusion, the corn-25(OH)D_3 premix provided 50 µg/kg of 25(OH)D_3 to the complete diet.

[4]The corn-phytase premix was prepared by mixing 333.33 g of phytase concentrate (1,500 phytase units per g; HiPhorius; DSM, Parsippany, NJ, USA) and 1,416.67 g ground corn. At

Table 3.1. (cont.)

0.50 % inclusion, the corn-phytase premix provided 500 units of microbial phytase to complete the diets.

[5]The vitamin-micromineral premix provided the calculated quantities of vitamins and micro minerals per kg of complete diet as following: vitamin A as retinyl acetate, 10,622 IU; vitamin D_3 as cholecalciferol, 1,660 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydroiodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

Table 3.2. Analyzed nutrient composition of diets and feed ingredients, as-fed basis[1]

Item	Ca and P:	Normal[2]	75% of requirements				Feed ingredients			
		PC[3]	NC[3]	NC	NC	NC				
	25(OH)D_3:	-	-	+	-	+		Soybean		Dicalcium
	Phytase:	-	-	-	+	+	Corn	meal	Limestone	phosphate
Dry matter, %		91.41	91.07	91.35	91.51	91.7	86.3	86.28	-	-
Gross energy, kcal/kg		4,011	4,013	4,010	4,035	4,013	3,918	3,937	-	-
Crude protein, %		16.88	16.81	16.94	16.93	17.31	7.11	45.44	-	-
Ash, %		4.30	3.81	3.92	3.78	3.70	4.87	4.32	99.84	88.26
Ca, %		0.63	0.49	0.51	0.49	0.50	0.03	0.33	38.12	19.96
P, %		0.58	0.49	0.52	0.49	0.51	0.31	0.71	0.22	18.96
Phytate[4], %		0.97	0.97	0.97	0.97	0.97	0.76	1.68	-	-
Phytate-P[5], %		0.27	0.27	0.27	0.27	0.27	0.21	0.47	-	-
Non-phytate P[6], %		0.31	0.22	0.25	0.22	0.24	0.10	0.24	-	-
Total dietary fiber[7], %		13.70	14.20	13.70	14.10	13.70	8.60	18.10	-	-
Soluble dietary fiber, %		2.00	2.00	2.00	1.80	1.60	0.40	2.20	-	-
Insoluble dietary fiber, %		11.70	12.20	11.70	12.30	12.10	8.20	15.90	-	-

Table 3.2. (cont.)

Phytase, units/kg	< 100	< 100	< 100	424	426	-	-	-	-
25(OH)D3 µg/kg	ND8	ND	43	ND	46	-	-	-	-

[1]Calculated metabolizable energy was 3,302 kcal/kg in the PC diet and 3,324 kcal/kg in the other diets. The calculated concentrations of standardized ileal digestible Lys, Met, and Thr were 0.98, 0.31, and 0.60%, respectively. The calculated concentrations of Ca and standardized total tract digestible P were 0.66 and 0.31 in the PC diet and 0.495 and 0.233% in all other diets.

[2]Normal = diets containing Ca and P at 100% of the requirement (NRC, 2012).

[3]PC = positive control; NC = negative control.

[4]Phytate in all diets was calculated based on the analyzed concentration of phytate in corn and soybean meal and the inclusion rate in each diet.

[5]Phytate-P was calculated by multiplying the analyzed phytate by 0.282 (Tran and Sauvant, 2004).

[6]Non phytate-P was calculated as total P minus phytate-P.

[7]Total dietary fiber was calculated as the sum of soluble dietary fiber and insoluble dietary fiber.

[8]ND = not detectable.

Table 3.3. Apparent total tract digestibility (ATTD) of dry matter (DM), and ATTD and standardized digestibility (STTD) of Ca and P in experimental diets fed to growing pigs[1]

Item	Ca and P:	Normal[2]	75% of requirements					Contrast *P*-value[4]		
		PC[3]	NC[3]	NC	NC	NC				
	25(OH)D_3:	-	-	+	-	+				
	Phytase:	-	-	-	+	+	SEM	PC vs. NC	25(OH)D_3	Phytase	Interaction
Feed intake, kg/d		1.31	1.31	1.29	1.28	1.30	0.03	0.814	0.796	0.226	0.073
Fecal excretion, kg/d		0.13	0.12	0.12	0.12	0.13	0.005	0.023	0.242	0.278	0.112
ATTD of DM, %		89.86	90.92	91.07	91.00	90.19	0.34	0.030	0.342	0.244	0.170
Ca intake, g/d		8.01	6.41	6.61	6.25	6.47	0.18	< 0.001	< 0.001	0.001	0.872
Ca excretion in feces, g/d		2.53	2.10	1.79	1.25	1.27	0.13	0.005	0.157	< 0.001	0.108
Absorbed Ca, g/d		5.47	4.31	4.79	4.99	5.19	0.13	< 0.001	0.006	< 0.001	0.226
ATTD of Ca, %		68.40	67.20	72.92	79.96	80.26	1.45	0.550	0.041	< 0.001	0.064
BEL[5] of Ca, mg/d		518	515	512	506	515	13.00	0.550	0.441	0.509	0.092
STTD[5] of Ca, %		74.87	75.23	80.65	88.07	88.23	1.45	0.857	0.056	< 0.001	0.072
P intake, g/d		7.62	6.41	6.66	6.28	6.60	0.18	< 0.001	< 0.001	0.083	0.547
P excretion in feces, g/d		3.46	3.30	2.87	1.93	2.19	0.15	0.313	0.439	< 0.001	0.003

Table 3.3. (cont.)

Absorbed P, g/d	4.16	3.11	3.76	4.34	4.39	0.12	< 0.001	0.007	< 0.001	0.019
ATTD of P, %	54.64	48.53	56.78	69.24	66.68	1.57	0.008	0.078	< 0.001	0.001
BEL of P[5], mg/d	227	226	225	222	226	6.00	0.550	0.442	0.509	0.093
STTD of P[5], %	57.62	52.05	60.15	72.78	70.11	1.57	0.015	0.093	< 0.001	0.001

[1]Each least squares mean represents 12 observations except for PC, NC, and the diet containing phytase and no 25(OH)D_3 (*n* = 11).

[2]Normal = diets containing Ca and P at 100% of the requirement (NRC, 2012).

[3]PC = positive control; NC = negative control.

[4]Four contrasts were used: PC vs. NC = effects of Ca and P levels in diets without 25(OH)D_3 or microbial phytase; 25(OH)D_3 = effects of 25(OH)D_3; phytase = effects of microbial phytase; and interaction = the interaction between 25(OH)D_3 and microbial phytase.

[5]BEL = basal endogenous loss; BEL was calculated by multiplying daily DM intake of pigs by BEL of Ca or P. Values for the STTD of Ca were calculated by correcting the ATTD of Ca with the average BEL of Ca (i.e., 433 mg/kg DM intake, Lee and Stein, 2023); values for the STTD of P were calculated by correcting the ATTD of P with the average BEL of Ca (i.e., 190 mg/kg DM intake; NRC, 2012).

Table 3.4. Concentration of osteocalcin (OC), bone alkaline phosphatase (BAP), carboxyterminal cross-linked telopeptide of type I collagen(CTX-I), and fibroblast growth factor (FGF23) in serum of growing pigs fed experimental diets for 13 days[1]

Item	Ca and P:	Normal[2]	75% of requirements					Contrast *P*-value[4]			
		PC[3]	NC[3]	NC	NC	NC	SEM	PC vs. NC	25(OH)D_3	Phytase	Interaction
	25(OH)D_3:	-	-	+	-	+					
	Phytase:	-	-	-	+	+					
OC, µg/L		38.08	36.17	39.20	40.17	42.90	1.47	0.150	0.005	< 0.001	0.880
BAP, µg/L		64.90	62.64	66.02	61.49	60.77	6.18	0.618	0.702	0.356	0.542
CTX-I, µg/L		0.14	0.13	0.14	0.13	0.12	0.04	0.735	0.926	0.817	0.645
OC to CTX-I ratio[5]		358	313	348	351	391	67	0.264	0.229	0.182	0.932
FGF23, µg/L		0.42	0.42	0.44	0.41	0.43	0.04	0.994	0.485	0.796	0.855

[1]Each least squares mean represents 12 observations except for PC, NC, and the diet containing phytase and no 25(OH)D_3 (n = 11).

[2]Normal = diets containing Ca and P at 100% of the requirement (NRC, 2012).

[3]PC = positive control; NC = negative control.

[4]Four contrasts were used: PC vs. NC = effects of Ca and P levels in diets without 25(OH)D_3 or microbial phytase; 25(OH)D_3 = effects of 25(OH)D_3; phytase = effects of microbial phytase; and interaction = the interaction between 25(OH)D_3 and microbial phytase.

Table 3.4. (cont.)

[5]OC to CTX-I ratio was calculated as the mean value of OC for the treatment divided by the mean value of CTX-I for the treatment (Lee et al., 2020).

Table 3.5. Vitamin D_3 metabolites in plasma of growing pigs fed experimental diets for 13 days[1]

Item	Ca and P:	Normal[2]	75% of requirements					Contrast *P*-value[4]		
		PC[3]	NC[3]	NC	NC	NC	SEM			
	25(OH)D_3:	-	-	+	-	+				
	Phytase:	-	-	-	+	+		PC vs. NC	25(OH)D_3	Phytase	Interaction
25(OH)D_3, ng/ml		17.50	13.38	44.98	19.36	49.90	1.80	0.121	< 0.001	0.007	0.771
24,25(OH)$_2$$D_3$, ng/ml		3.47	1.57	8.55	3.97	10.49	0.63	0.011	< 0.001	< 0.001	0.636
VMR[5]		19.76	11.89	18.87	20.30	21.02	2.16	0.001	0.016	0.002	0.041
1,25(OH)$_2$$D_3$, pg/ml		166.10	270.18	291.38	211.60	218.88	15.30	< 0.001	0.363	< 0.001	0.654

[1]Each least squares mean represents 5 observations.

[2]Normal = diets containing Ca and P at 100% of the requirement (NRC, 2012).

[3]PC = positive control; NC = negative control.

[4]Four contrasts were used: PC vs. NC = effects of Ca and P levels in diets without 25(OH)D_3 or microbial phytase; 25(OH)D_3 = effects of 25(OH)D_3; phytase = effects of microbial phytase; and interaction = the interaction between 25(OH)D_3 and microbial phytase.

[5]VMR = vitamin D metabolite ratio; 24,25(OH)$_2$$D_3$ / 25(OH)D_3 × 100 (Zelzer et al., 2020).

Table 3.6. Percentage changes of vitamin D_3 metabolites in plasma of growing pigs from day 1 to day 13[1]

Item	Ca and P:	Normal[2]	75% of requirements					Contrast *P*-value[4]		
		PC[3]	NC[3]	NC	NC	NC				
	25(OH)D_3:	-	-	+	-	+				
	Phytase:	-	-	-	+	+	SEM	PC vs. NC	25(OH)D_3	Phytase	Interaction
25(OH)D_3, %		40.1	46.4	250.7	118.8	299.5	39.5	0.902	< 0.001	0.160	0.780
24,25$(OH)_2D_3$, %		61.1	18.4	359.3	130.1	378.7	40.1	0.411	< 0.001	0.136	0.287
VMR[5]		13.6	-26.1	31.0	9.5	20.7	10.8	0.009	0.007	0.281	0.057
1,25$(OH)_2D_3$, %		-33.2	8.5	23.4	-7.1	-12.4	7.8	0.001	0.540	0.009	0.225

[1]Each least squares mean represents 10 observations on day 1, and 5 observations on day 13. Day 1 baseline. Day 13 is de new value (i.e., [(Day 13 – Day 1)/Day 1] *100).

[2]Normal = diets containing Ca and P at 100% of the requirement (NRC, 2012).

[3]PC = positive control; NC = negative control.

[4]Four contrasts were used: PC vs. NC = effects of Ca and P levels in diets without 25(OH)D_3 or microbial phytase; 25(OH)D_3 = effects of 25(OH)D_3; phytase = effects of microbial phytase; and interaction = the interaction between 25(OH)D_3 and microbial phytase.

[5]VMR = vitamin D metabolite ratio; 24,25$(OH)_2D_3$ / 25(OH)D_3 × 100 (Zelzer et al., 2020)

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CHAPTER 4: Effects of graded levels of phytase on apparent total tract digestibility of energy and concentrations of digestible and metabolizable energy in soybean meal

Abstract

The objective of this experiment was to test the hypothesis that digestible energy (**DE**) and metabolizable energy (**ME**) in soybean meal (**SBM**) is increased by microbial phytase. The second hypothesis was that the source of soybean meal does not impact the response of growing pigs to microbial phytase. Three sources of SBM from different locations in the United States, were labeled SBM 01, SBM 02, and SBM 03, respectively. A total of 128 growing pigs (initial body weight: 14.29 ± 1.9 kg) were housed individually in metabolism crates and assigned to a randomized complete block design, with 4 blocks, 16 diets, and 8 replicate pigs per diet. Each source of SBM was included in 5 corn-SBM diets containing 0, 500, 1,000, 2,000, or 4,000 units of phytase per kilo of diet and a corn-based diet was also used. Feces and urine were collected to determine apparent total tract digestibility (**ATTD**) of energy in all diets, and to calculate DE and ME in all diets. The DE and ME in each source of SBM were calculated by difference. The ATTD and standardized total tract digestibility (**STTD**) of Ca and P were also calculated for all diets. Results demonstrated that source of SBM had no influence on DE and ME in the corn-SBM diets or in the SBM ingredients. Phytase supplementation increased (quadratic, $P < 0.01$) ATTD of dry matter and ATTD of gross energy also increased (linear, $P \leq 0.05$). Consequently, DE and ME of diets increased (quadratic, $P < 0.01$ and $P < 0.05$, respectively), and DE:GE increased (linear, $P < 0.05$) with increasing phytase in diets. Likewise, ATTD of gross energy in SBM increased (linear, $P < 0.01$) and DE and ME in SBM increased (quadratic, $P < 0.01$ and $P < 0.05$, respectively) as phytase inclusion increased. Phytase level also increased (quadratic, $P < 0.001$) ATTD and STTD of P and Ca, whereas, the daily basal endogenous losses of Ca and P

were not affected. In conclusion, microbial phytase improves energy utilization and mineral digestibility in SBM and ME in SBM is increased by 40 to 50 kcal/kg if microbial phytase is used. The lack of difference among SBM sources indicates that the response to phytase on digestibility of energy, Ca and P likely us independent of the source of SBM.
Key words: calcium, digestible energy, metabolizable energy, microbial phytase, phosphorus, soybean meal

Abbreviations: AEE, acid hydrolyzed ether extract; ATTD, apparent total tract digestibility; DE, digestible energy; DM, dry matter; FTU, phytase units; GE, gross energy; IDF, insoluble dietary fiber; ME, metabolizable energy; SBM, soybean meal; SDF, soluble dietary fiber; STTD, standardized total tract digestibility; TDF, total dietary fiber.

Introduction

Soybean meal (**SBM**) is a high protein ingredient with a favorable amino acid profile that is often used in swine diets (Kudelka et al., 2021). In addition to its high protein content, SBM contributes to the energy in diets for pigs and the metabolizable energy (**ME**) in SBM is greater than in corn for growing pigs and sows (Sotak-Peper et al., 2015; Kim et al., 2025). Soybean meal also contains P, but the majority of P in SBM is bound to phytate as is the case for most plant-based feed ingredients (Lee et al., 2023). Therefore, diets for pigs are usually fortified with microbial phytase, which increases the digestibility of P from SBM and other ingredients (Rojas and Stein, 2012; Almeida et al., 2017).

Whereas the impact of phytase on the digestibility of P is well documented, there is a lack of information about the impact of phytase on the digestibility of energy. It has been hypothesized that phytate may chelate starch and amino acids in the intestinal tract (Selle and Ravindran, 2008; Selle et al., 2009), and if that is correct, it is likely that addition of phytase to

the diets can release energy containing nutrients and thereby increase digestibility of energy (Lagos et al., 2022). Energy digestibility in corn–SBM diets has been reported to increase in some experiments if microbial phytase was used (Liao et al., 2005; Arredondo et al., 2019; Lala et al., 2020; Espinosa et al., 2022), but not in others (She et al., 2018; Lamp and Moritz, 2022; Lagos et al., 2022), and it is not clear why different responses are obtained among experiments. However, SBM contains more phytate than corn (Nelson et al., 1968; Lee et al., 2023a), and inclusion of phytase in diets containing SBM may, therefore, reduce the possible negative effects of phytate on digestibility of amino acids, which may result in an increased digestibility of not only P, but also of energy. There is, however, a lack of information about the impact of microbial phytase on the digestibility of energy in SBM, and to our knowledge, the impact of graded levels of microbial phytase on digestibility of energy in SBM has not been reported. It is also not known if a possible effect of microbial phytase on energy digestibility in SBM is consistent among different sources of SBM.

Therefore, an experiment was conducted to test the hypothesis that inclusion of graded levels of microbial phytase in corn-SBM diets fed to growing pigs will increase the apparent total tract digestibility (**ATTD**) of energy and the concentration of digestible energy (**DE**) and ME in SBM. The second hypothesis was that the impact of microbial phytase on digestibility of energy in SBM is consistent among different sources of SBM.

Materials and methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment before animal work was initiated. Pigs used in the experiment were the offspring of Line 800 boars and Camborough females (PIC, Hendersonville, TN, USA).

One hundred and twenty-eight growing pigs with an average initial BW of 14.29 ± 1.9 kg were used. Three sources of SBM were procured from J&R distributing Inc., Lake Norden, SD, USA; Archer Daniels Midland Company, Decatur, IL,USA; and United Animal Health, Sheridan, IN, USA, respectively (Table 4.1). The 3 sources were randomly labelled SBM 01, SBM 02, and SBM 03. Each source of SBM was included in 5 corn-SBM diets containing 0, 500, 1,000, 2,000, or 4,000 phytase units **(FTU)** per kilo of diet (Quantum Blue, AB Vista, Marlborough, UK) and a corn-based diet was also formulated (Tables 4.2 and 4.3). Therefore, a total of 16 diets were used. Pigs were housed individually in metabolism crates (0.67 × 0.80 m) that were equipped with a slatted floor, a feeder, and a nipple drinker. A screen floor for total fecal collection and a urine pan were installed below the slatted floor, which allowed for separate collection of feces and urine.

Pigs were allotted to the 16 diets using a randomized complete block design with 4 blocks of 32 pigs (2 pigs per diet in each block) for a total of 8 replicate pigs per diet (Kim and Lindemann, 2007). Feed was provided daily in the amount of 3.5 times the energy requirement for ME based on the initial body weight of pigs (i.e., 197 kcal ME/kg of body weight$^{0.60}$; NRC, 2012). Water was available at all times. The daily allotment of feed was divided in 2 equal meals and provided at 0800 and 1600 h. Diets were fed for 12 days. The initial 5 days were the adaptation period to the diets, which was followed by 4 days of collection of feces and urine

according to the marker-to-marker approach (Adeola, 2001). In short, on day 6, a color marker (i.e., indigo carmine) was added to the morning meal to mark the beginning of fecal collection, and the stop marker (i.e., ferric oxide) was added to the morning meal on day 10 to mark the conclusion of fecal collection. Fecal samples were stored at -20°C immediately after collection. Urine was collected in urine buckets over a preservative of 50 mL of hydrochloric acid, starting at 0900 h on day 6 and ceased at 0900 h on day 10. On each collection day, 10% of the collected urine was stored at −20 °C until analyzed. Orts collected during the collection period were dried at 65°C in a forced air oven (Heratherm OMH750; Thermo Fisher 1873 Scientific Inc., Waltham, MA, USA) and the dried weight was subtracted from the total provision of diet that was fed to calculate feed consumption after correction for dry matter (Adeola, 2001). At the conclusion of the experiment, fecal samples were dried as explained for the orts and ground though a 1-mm screen using a grain mill (model: RRH-500, Zhejiang Winki Plastic Industry Co., Ltd., Zhejiang, China). Urine samples were thawed, filtered, and mixed, and a subsample was lyophilized (Kim et al., 2009).

Chemical analysis

Corn and SBM were analyzed for phytate (Ellis et al., 1977). Phytase activity in diets was analyzed according to method 2000.12 (AOAC Int., 2019). Diets, corn, SBM, feces, and urine samples were analyzed for gross energy (**GE**) using bomb calorimetry (Model 6400, Parr Instruments, Moline, IL, USA). Corn, SBM, diets, and fecal samples were analyzed for DM **(DM;** method 930.15; AOAC Int., 2019) and ash (method 942.05; AOAC Int., 2019). Concentrations of Ca and P in corn, SBM, diets, and dried fecal samples were also analyzed (method 985.01 A, B, and C; AOAC Int., 2019) using inductively coupled plasma-optical emission spectrometry (Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation for

Ca and P included dry ashing at 600 °C for 4 h according to method 942.05 (AOAC Int., 2019) and wet digestion with nitric acids according to method 3050 B (U.S.-EPA, 2000). In corn and the 3 sources of SBM, Mg, K, Na, Cu, Fe, Mn, and Zn were analyzed using the same procedure as used to analyze Ca and P. Corn, SBM, and diets were also analyzed for acid hydrolyzed ether extract (**AEE**; method 2003.06; AOAC Int., 2019; Ankom XT15 Extractor; Ankom Technology, Macedon, NY). Insoluble dietary fiber and soluble dietary fiber were analyzed in diets, corn, and SBM using the Ankom Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA; method 991.43, AOAC Int., 2019). Total dietary fiber (**TDF**) was calculated as the sum of soluble dietary fiber and insoluble dietary fiber. Crude protein was calculated as analyzed nitrogen × 6.25 and nitrogen in diets, corn, and SBM was analyzed by combustion (method 990.03; AOAC Int., 2019) using a LECO FP628 Nitrogen Analyzer (LECO Corp., Saint Joseph, MI, USA). Glucose, fructose, maltose, and sucrose were analyzed in corn and SBM; and stachyose, and raffinose were analyzed in the 3 sources of SBM, using high performance liquid chromatography (Dionex Tech. Notes 21 & 92, Sunnyvale, CA, USA, Navarro et al., 2018). Soybean meal was also analyzed for trypsin inhibitors (method Ba 12-75; AOCS, 2006).

Calculations and statistical analysis

Phytate-bound P in diets and ingredients was calculated by multiplying the analyzed phytate by 0.282 (Tran and Sauvant, 2004) and non-phytate P was calculated by subtracting phytate-bound P from total P. The DE (kcal/kg) and ME (kcal/kg) in diets were calculated by subtracting GE in feces (kcal/d), and GE in feces (kcal/d) and urine (kcal/d), respectively, from the intake of GE (kcal/d; NRC, 2012). The DE and ME in corn were calculated by dividing DE and ME in the corn diet by the inclusion rate of corn in that diet (i.e., 96.3%). The contribution of DE and ME from corn to the DE and ME in the corn-SBM diets was then calculated, which

allowed for the calculation of DE and ME in SBM in each of the corn-SBM diets using the difference procedure (Adeola, 2001). The ATTD of DM, GE, Ca and P was calculated for all diets and for the 3 sources of SBM, using the following equation (Almeida and Stein, 2010):

$$\text{ATTD (\%)} = [(\text{intake} - \text{output}) \div \text{intake}] \times 100.$$

The standardized total tract digestibility (**STTD**) of P (%) and Ca (%) was calculated using the following equation (Almeida and Stein, 2010):

$$\text{STTD (\%)} = \{[\text{intake} - (\text{output} - \text{daily basal endogenous loss})] \div \text{intake}\} \times 100,$$

where intake, output, and daily basal endogenous loss of P and Ca are in grams per day. The basal endogenous loss of P was assumed to be 190 mg/kg DM intake (NRC, 2012), and basal endogenous loss of Ca was assumed to be 433 mg/kg DM intake (Lee and Stein, 2023).

Data were analyzed using mixed procedures in SAS (SAS Inst. Inc., Cary, NC, USA). Outliers were determined by plotting the residuals in a quantile-quantile plot against the normal distribution and identifying values that were beyond ± 3.0 standard deviations. Normality and homogeneity of data were verified using the UNIVARIATE and MIXED procedures in SAS. Treatment means were calculated using the LSMEANS statement in SAS. The initial model included SBM source, phytase, and the interaction between SBM source and phytase as fixed variables and block and replicate within block as random variables. However, no interactions between SBM source and phytase were observed, and the final model, therefore, contained only source of SBM and phytase. Contrast statements were used to determine linear and quadratic effects of increasing phytase in diets. Unequally spaced contrasts were calculated using the PROC IML procedure of SAS. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Pigs remained healthy during the experiment and no pigs had to be removed from the experiment. Pigs easily consumed their diets and limited feed refusals were observed. Ingredients and diets were analyzed before the animal part of the experiment was initiated and correct diet mixing was confirmed.

Energy digestibility

Pigs fed diets containing SBM 01 tended ($P < 0.10$) to have greater daily feed intake than pigs fed diets containing SBM 03, and GE intake was greater ($P < 0.05$) for pigs fed diets containing SBM source 01 compared with pigs fed a diet containing SBM source 03 (Table 4.4). However, fecal output and fecal GE excretion were not affected by source of SBM. Likewise, urine output and urine GE excretion did not differ among treatments. The ATTD of DM and GE in the complete diets was also not impacted by source of SBM. The DE in diets with SBM 01 tended ($P < 0.10$) to be greater than in diets with SBM 03, but there were no differences among sources of SBM for ME, or DE to GE, ME to DE, or ME to GE.

Phytase supplementation did not affect feed intake or GE intake of pigs. Fecal output decreased (quadratic, $P < 0.01$) and GE excretion in feces tended to decrease (quadratic, $P < 0.10$) as phytase inclusion increased. In contrast, urine output and urine GE output tended to increase (linear, $P < 0.10$) with increasing phytase inclusion. The ATTD of DM in diets increased (quadratic $P < 0.01$) with increasing phytase inclusion, and ATTD of GE also increased (linear, $P \leq 0.05$) as phytase inclusion increased. The DE and ME of diets increased (quadratic, $P < 0.01$, and $P < 0.05$, respectively) with increasing phytase supplementation, and DE to GE increased as well (linear, $P < 0.05$).

The ATTD of GE and DE tended to be greater ($P < 0.10$) for SBM 01 than for SBM 03, but ME was not different among the 3 sources of SBM (Table 4.5). The ATTD of GE in SBM increased ($P < 0.01$) as phytase inclusion increased, and DE and ME in SBM also increased (quadratic, $P < 0.01$ and 0.05, respectively) with increasing phytase. Likewise, DE to GE and ME to GE increased (quadratic, $P < 0.05$) as dietary phytase increased.

Ca and P digestibility

The intake of P tended ($P < 0.10$) to be greater for pigs fed diets containing SBM 01 compared with pigs fed diets containing SBM 03 (Table 4.6). Fecal P excretion was not affected by SBM source, whereas absorbed P was greater ($P < 0.01$) for pigs fed diets containing SBM 01 compared with SBM 03. The ATTD and STTD of P tended ($P < 0.10$) to be greater for pigs fed SBM 02 than for pigs fed SBM 03, and the basal endogenous loss tended ($P < 0.10$) to be greater for pigs fed a diet containing SBM 01 than pigs fed a diet with SBM 03.

Phytase supplementation did not affect P intake, but increasing phytase in diets reduced (quadratic, $P < 0.001$) the concentration of P in the feces and fecal P excretion and increased (quadratic, $P < 0.001$) absorbed P. The ATTD and STTD of P also increased (quadratic, $P < 0.001$) by phytase addition to diets, whereas basal endogenous loss of P was not affected by increased phytase in diets.

The daily intake of Ca tended ($P < 0.10$) to be greater by pigs fed SBM 01 than pigs fed SBM 03. The concentration of Ca in feces was greater ($P < 0.001$) for pigs fed SBM 03 or SBM 02 compared with SBM 01, and daily fecal excretion was also greater ($P < 0.05$) for pigs fed SBM 02 or SBM 03 compared with SBM 01. Absorbed Ca was greater ($P < 0.001$) for pigs fed SBM 01 or SBM 02 compared with SBM 03. The ATTD and STTD of Ca were greater ($P < 0.001$) for pigs fed SBM 01 than for pigs fed SBM 03, whereas the daily basal endogenous loss

of Ca tended ($P < 0.10$) to be greater for pigs fed a diet containing SBM 01 compared with pigs fed a diet containing SBM 03. Intake of Ca was not affected by dietary phytase, but Ca in feces and fecal excretion of Ca decreased (quadratic, $P < 0.001$) as dietary phytase supplementation increased. Therefore, absorbed Ca increased (quadratic, $P < 0.001$) and ATTD and STTD of Ca also increased (quadratic, $P < 0.001$) as dietary phytase levels increased. The basal endogenous loss of Ca was not affected by phytase supplementation.

Discussion

Analyzed phytase in all diets was in agreement with formulated values. Crude protein ranged from 45.08 to 47.37%, and sucrose ranged from 5.91 to 7.08% in the 3 sources of SBM. Phytic acid concentration ranged from 1.42 to 1.51%, which is slightly less than previously reported (Tahir et al., 2012; Lee et al., 2023). Calcium in the 3 sources of SBM ranged from 0.20 to 0.57%, which is also within the range of reported values (Sotak- Peper et al., 2015; Lee et al., 2023).

The observation that there were no interactions between source of SBM and phytase indicates that all sources had a similar response to phytase which is in agreement with previous data (Sotak-Peper et al., 2016). The observation that STTD of Ca and P increased linearly as phytase inclusion increased in diets demonstrates that phytase worked as expected. This response is consistent with phytate being an anti-nutritional factor that reduces Ca and P digestibility through binding and formation of a Ca-P phytate complex. Use of phytase in diets for pigs, therefore, consistently improves the digestibility of minerals when phytase is added to pig diets and ingredients (Selle et al., 2009; Almeida and Stein, 2012; Arredondo et al., 2019; Hu et al., 2023).

Because of the increased digestibility of Ca and P, it was concluded that the phytase used facilitated hydrolysis of the ester bonds attaching P to the inositol ring in phytase. In addition to the release of Ca and P from inositol it is also possible that protein/amino acids and possibly starch were bound to the phytate complex, which may have reduced digestion and increased losses of amino acid and energy (Selle et al., 2000; Ravindran et al., 2001; Arredondo et al., 2019; Dersjant-Li and Dusel, 2019). If binding of starch and amino acids takes place, it is expected that use of phytase will increase digestibility of these nutrients, and therefore also of energy. Indeed, increased ileal digestibility of amino acids as a result of adding microbial phytase to corn-SBM diets has been demonstrated (Espinosa et al., 2022; Lagos et al 2022; 2023), and ileal digestibility if starch has also been increased by microbial phytase (Espinosa et al., 2022). Therefore, digestibility of energy in corn-SBM diets has sometimes been increased by phytase (Liao et al., 2005; Arredondo et al., 2019; Lala et al., 2020; Espinosa et al., 2021). However, to our knowledge no data demonstrating the impact of phytase on DE and ME in SBM have been published. Therefore, the observation that DE and ME increased with phytase in SBM indicates that some energy containing nutrients in SBM were bound to phytate and thus reduced ATTD of energy, but phytase reduced that negative effect, and the bound nutrients could be absorbed if phytase was used. Because SBM is high in amino acids and does not contain starch it is likely that microbial phytase released amino acids from phytate, which then increased DE and ME. This hypothesis is in agreement with observations of "extra-phosphoric" effects of phytase (Liao et al., 2005; Selle et al., 2009) and aligns with results of experiment where phytase increased energy utilization in corn–SBM diets (Arredondo et al., 2019; Park et al., 2026).

Although increased energy digestibility in corn-SBM diets has been previously demonstrated, there are also data from experiments in which no increase was observed (She et

al., 2018; Mesina et al., 2019; Lamp and Moritz, 2022; Adeshakin et al., 2025). It is not clear what the reason for these different responses are. It is unlikely that the relatively short length of digestibility experiment influences the impact of phytase on digestibility of amino acids and starch because as demonstrated in this experiment, the action of phytase on mineral digestibility is apparent after 5 days of adaptation. It is also unlikely that the reason for lack of consistency is differences among phytases because increase in Ca and P digestibility indicates destruction of the phytate complex, which theoretically should release any bound amino acids or starch. In addition, the phytase that was used in this present experiment is identical to the phytase used previously in experiment where no impact of phytase on energy digestibility was observed (Lagos et al., 2022; 2023).

The efficiency of phytase in degrading phytate may be influenced by pig age and appears to be greater in younger pigs (Cambra-López et al., 2020; Lagos et al., 2022). In the present experiment, pigs with a starting weight of 14 kg were used, which may have contributed to the responses obtained. Because the response to phytase also may depend on diet composition and P level in the diets, the high SBM inclusion used in the present experiment may have contributed to the energy response observed (Almeida et al., 2013).

In addition to release of minerals, amino acids, and starch, phytase will also result in release of inositol, which can be absorbed from the small intestine, and therefore increase plasma inositol (Lagos et al., 2023; Park et al., 2026). Pigs can synthetize inositol, but they need glucose to do so and absorption of inositol may therefore spare the energy needed for inositol synthesis. Absorption of inositol may also enable pigs to maintain greater plasma inositol levels than if fed a diet without phytase (Moran et al., 2019; Lagos et al., 2021; Guan et al., 2025; Mallea et al., 2025), which may have benefits to maintaining the immune system and therefore spare energy.

However, because we did not measure immune parameters in this experiment, we cannot confirm this hypothesis.

The observation that DE and ME of SBM without phytase were slightly lower than reference values (NRC, 2012) is a result of the chemical composition of the SBM used in the present work. In particular, crude protein and sucrose were somewhat less than typical values for SBM, and both components contribute to the energy value of SBM. The use of 14 kg pigs likely also contributed to the reduced DE and ME in SBM because younger have reduced ATTD of GE than older pigs (Noblet et al., 1994; Kil et al., 2013; Lagos et al., 2022).

The increase in DE and ME with phytase that was observed indicates that DE and ME in SBM may increased from 3,349 to 3,455 kcal of ME per kilo if 2,000 FTU/kg of microbial phytase is including in the diet. Because there was no SBM source by phytase interaction, the same increase in DE and ME can likely be applied to all sources of SBM. As a consequence, in addition to the increased digestibility of Ca and P that is obtained by phytase, the energy value of SBM is also increased.

Conclusions

Results demonstrated that in addition to an improvement in STTD of Ca and P in SBM, microbial phytase also releases energy- containing nutrients that will result in an increase in DE and ME. Results indicate that the source of SBM does not impact the response to microbial phytase, but an increase from 3,349 to 3,455 kcal if 2,000 FTU/kg of ME per kg SBM, may be achieved if microbial phytase is added to diets for young growing pigs.

Disclosures

The authors have no conflicts of interest.

Tables

Table 4.1. Analyzed composition of corn, and three sources of soybean meal

Item	Corn	Soybean meal		
		01	02	03
Dry matter, %	87.95	88.45	88.32	88.82
GE, kcal/kg	3,918	4,136	4,119	4,135
Crude protein, %	7.53	47.37	45.08	46.50
AEE[1], %	2.67	2.41	2.14	2.75
Ash, %	1.32	5.71	6.13	6.43
Phytate, %	0.79	1.51	1.50	1.42
Ca, %	0.01	0.26	0.47	0.57
P, %	0.306	0.69	0.69	0.67
Phytate-bound P[2]	0.22	0.43	0.42	0.40
Non-phytate-P[3]	0.49	1.08	1.08	1.02
TDF[1], %	9.90	18.10	15.90	16.90
IDF[1], %	9.20	17.60	15.30	16.20
SDF[1], %	0.70	0.50	0.60	0.70
Mg, %	0.35	0.26	0.29	0.26
K, %	0.01	2.06	1.98	1.81
Na, %	0.10	0.04	0.07	0.08
Cu, mg/kg	122.50	24.65	19.40	79.30
Fe, mg/kg	3.00	164.13	113.88	503.29
Mn, mg/kg	18.73	55.80	43.85	193.74
Zn, mg/kg	4.91	72.20	67.58	566.38

Table 4.1. (cont.)

Glucose	0.66	<0.05	<0.05	<0.05
Maltose	1.14	<0.05	<0.05	<0.05
Fructose	0.23	<0.05	<0.05	0.08
Sucrose	0.40	6.63	7.08	5.91
Stachyose	-	5.36	4.34	4.90
Raffinose	-	1.12	1.67	1.05
Trypsin inhibitor, units/g	-	4,180	3,050	2,090

[1]AEE = acid-hydrolyzed ether extract; TDF = total digestible fiber; SDF = soluble digestible fiber; IDF = insoluble digestible fiber.

[2]Phytate-bound P was calculated by multiplying the analyzed phytic acid by 0.282 (Tran and Sauvant, 2004).

[3]Nonphytate-P was calculated as the difference between total P and phytate-P.

Table 4.2. Ingredient composition of experimental diets[1], as-fed basis

		Soybean meal		
Item, %	Corn	01	02	03
Corn	96.29	57.40	57.40	57.40
Soybean meal 01	-	40.00	-	-
Soybean meal 02	-	-	40.00	-
Soybean meal 03	-	-	-	40.00
Dicalcium phosphate	1.55	0.40	0.40	0.40
Calcium carbonate	0.80	0.80	0.80	0.80
Phytase premix[2]	0.50	0.50	0.50	0.50
Vitamins-mineral premix[3]	0.50	0.50	0.50	0.50
Salt	0.40	0.40	0.40	0.40

[1] For each source of soybean meal, there was one diet without phytase and 4 diets containing 500, 1,000, 2,000, or 4,000 units of phytase, per kg of diet.

[2]Five separate premixes were prepared to provide 0, 500, 1,000, 2,000, or 4,000 units of phytase per kg of final diets. Premixes were prepared by mixing ground corn (100, 98, 96, 92, or 84%) and 0, 2, 4, 8, or 16% of the phytase concentrate (Quantum Blue, AB Vista, Marlborough, UK; 5,000 phytase units per g).

[3]The vitamin-micromineral premix provided the calculated quantities of vitamins and micro minerals per kg of complete diet as following: vitamin A as retinyl acetate, 10,622 IU; vitamin D_3 as cholecalciferol, 1,660 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; $_{D}$-

Table 4.2. (cont.)

pantothenic acid as $_{D}$-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydroiodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

Table 4.3. Analyzed nutrient composition of diets, as-fed basis[1]

Item, %	Corn diet	Soybean meal			Soybean meal			Soybean meal			Soybean meal			Soybean meal		
		01	02	03	01	02	03	01	02	0.3	01	02	03	01	02	03
Phytase, units/kg		0 FTU[1]/kg			500 FTU/kg			1,000 FTU/kg			2,000 FTU/kg			4,000 FTU/kg		
Dry matter, %	88.05	88.21	88.47	88.59	88.8	88.97	89.45	88.74	89.43	88.65	89.25	88.49	88.44	88.95	88.89	88.88
GE, kcal/kg	3,762	3,950	3,925	3,892	3,915	3,895	3,893	3,895	3,938	3,915	3,929	3,901	3,901	3,864	3,871	3,874
Crude protein, %	7.24	23.56	20.34	22.11	23.14	20.33	22.17	23.67	20.55	21.93	23.76	20.37	22.67	24.01	20.12	22.27
Ash, %	3.40	4.54	4.60	4.71	4.42	4.50	4.70	4.28	4.36	4.66	4.38	4.32	4.39	4.41	4.59	4.79
AEE[1], %	3.70	2.76	2.93	2.86	2.49	3.60	3.33	3.08	3.29	3.19	2.90	3.43	2.98	2.39	2.77	2.56
Ca, %	0.71	0.68	0.72	0.72	0.60	0.70	0.70	0.56	0.61	0.71	0.56	0.57	0.58	0.55	0.61	0.72
P, %	0.56	0.57	0.53	0.52	0.51	0.51	0.47	0.50	0.52	0.47	0.50	0.49	0.49	0.47	0.50	0.50
Phytase, units/kg	<70	<70	<70	<70	550	490	370	1,200	1,200	1,300	2,700	2,200	2,100	4,300	5,300	5,300
TDF[1], %	11.6	12.50	13.72	13.40	12.95	14.25	13.85	12.39	13.60	13.04	13.45	13.40	15.70	12.55	13.99	13.85
SDF[1], %	2.79	2.55	2.70	2.50	2.02	2.85	2.55	2.44	2.72	2.25	2.45	2.15	2.55	2.02	2.85	2.55
IDF[1], %	8.81	11.63	11.02	10.80	10.93	11.40	11.30	9.95	10.88	10.79	11.73	11.43	11.63	10.53	11.14	11.30

[1]FTU = phytase units per kilogram of diet; AEE = acid-hydrolyzed ether extract; TDF = total dietary fiber; SDF = soluble dietary fiber; IDF = insoluble dietary fiber.

Table 4.4 Apparent total tract digestibility (ATTD) of dry matter (DM) and gross energy (GE) and concentrations of digestible energy (DE) and metabolizable energy (ME) of corn-soybean meal diets, containing 0, 500, 1,000, 2,000 or 4,000 units of phytase per kilogram of diet fed to growing pigs.[1]

Item, %	Soybean meal, source			SEM	*P*-value	Phytase, phytase unit/kg					SEM	*P*-value	
	01	02	03		SBM	0	500	1,000	2,000	4,000		Linear	Quadratic
Intake													
Diet, g/day	967[x]	952[xy]	928[y]	23.72	0.056	941	950	946	944	966	25.51	0.277	0.635
GE, kcal/d	3,782[a]	3,721[ab]	3,616[b]	92.45	0.032	3,680	3,708	3,704	3,691	3,749	99.37	0.449	0.810
Fecal excretion													
Dry feces output, g/d	98.14	98.84	97.67	7.58	0.926	102.72	104.51	100.36	88.04	95.44	7.78	0.003	0.008
GE, kcal/d	471	471	464	33.67	0.866	473	495	481	431	464	34.63	0.089	0.086
Urine excretion													
Urine output, kg/d	6.69	6.43	5.76	1.11	0.285	5.89	5.68	6.31	6.61	6.98	1.16	0.077	0.746
GE in urine, kcal/d	170	163	149	10.08	0.121	156	152	160	160	174	11.69	0.091	0.752
Diet													
ATTD of DM, %	89.32	89.06	88.84	0.51	0.272	88.47	88.49	88.79	90.08	89.54	0.53	< 0.001	0.009
ATTD of GE, %	87.58	87.37	87.16	0.67	0.427	87.16	86.72	87.00	88.34	87.64	0.70	0.016	0.073

Table 4.4 (cont.)

DE, kcal/kg	3,425[x]	3,413[xy]	3,396[y]	26.16	0.070	3,409	3,383	3,407	3,456	3,402	27.15	0.351	0.004
ME, kcal/kg	3,249	3,243	3,234	28.36	0.732	3,243	3,223	3,235	3,287	3,222	30.30	0.909	0.038
Diet energy efficiency, %													
DE to GE	87.58	87.37	87.16	0.67	0.427	87.16	86.72	87.00	88.34	87.64	0.70	0.016	0.073
ME to DE	94.86	95.00	95.24	0.24	0.529	95.12	95.28	94.96	95.10	94.7	0.31	0.242	0.749
ME to GE	83.08	83.01	83.02	0.73	0.984	82.92	82.62	82.63	84.02	83.00	0.77	0.384	0.165

[a-b] Means within a row lacking a common subscript letter are different ($P < 0.05$). [x-y] Means within a row lacking a common subscript letter tend to be different ($P < 0.10$).

[1] Interactions between source of soybean meal and level of phytase were not significant. Therefore, only main effects are presented. Data are least squares means; n = 40 for soybean meal sources and n = 24 for phytase levels.

Table 4.5. Apparent total tract digestibility (ATTD) of dry matter (DM) and gross energy (GE) and concentrations of digestible energy (DE) and metabolizable energy (ME) of soybean meal, containing 0, 500, 1,000, 2,000, or 4,000 units of phytase per kilogram of diet fed to growing pigs.[1]

Item, %	Soybean meal, source			SEM	*P*-value	Phytase, phytase unit/kg					SEM	*P*-value	
	01	02	03		SBM	0	500	1,000	2,000	4,000		Linear	Quadratic
Ingredient													
ATTD of GE, %	88.3[x]	87.97[xy]	86.56[y]	1.58	0.056	87.46	85.89	87.32	90.32	87.06	1.64	0.351	0.004
DE, kcal/kg	3,652[x]	3,624[xy]	3,579[y]	65.39	0.070	3,612	3,547	3,606	3,730	3,595	67.86	0.352	0.004
ME, kcal/kg	3,360	3,344	3,323	70.90	0.732	3,344	3,295	3,326	3,455	3,293	75.76	0.909	0.038
Ingredient energy efficiency, %													
DE to GE	98.33[x]	97.8[xy]	96.34[y]	1.79	0.065	98.35	94.88	96.82	100.77	96.62	1.85	0.656	0.019
ME to DE	91.98	92.26	92.76	0.59	0.623	92.55	92.9	92.12	92.57	91.54	0.75	0.262	0.719
ME to GE	81.23	81.19	80.37	1.72	0.687	80.97	79.78	80.53	83.66	79.72	1.83	0.909	0.038

[a-b] Means within a row lacking a common subscript letter are different ($P < 0.05$). [x-y] Means within a row lacking a common subscript letter tend to be different ($P < 0.10$).

[1] Interactions between source of soybean meal and level of phytase were not significant. Therefore, only main effects are presented. Data are least squares means; n = 40 for soybean meal sources and n = 24 for phytase levels. `

Table 4.6. Apparent total tract digestibility (ATTD) and standardized digestibility (STTD) of Ca and P in corn-SBM diets containing three different sources of SBM and 0, 500, 1,000, 2,000 or 4,000 units of phytase per kilogram diet fed to growing pigs.[1]

Item	Soybean meal, source			SEM	*P*-value	Phytase, phytase unit/kg					SEM	*P*-value	
	01	02	03		SBM	0	500	1,000	2,000	4,000		Linear	Quadratic
P digestibility													
P intake, g/day	5.03[x]	4.95[xy]	4.83[y]	0.12	0.056	4.89	4.94	4.92	4.91	5.02	0.13	0.277	0.635
P in feces, %	1.52	1.51	1.56	0.05	0.396	2.35	1.59	1.54	1.18	0.99	0.06	< 0.001	< 0.001
Fecal P excretion, g/day	1.51	1.47	1.54	0.08	0.526	2.39	1.66	1.52	1.03	0.94	0.09	< 0.001	< 0.001
Absorbed P, %	3.52[a]	3.48[ab]	3.29[b]	0.09	0.008	2.50	3.29	3.40	3.87	4.08	0.10	< 0.001	< 0.001
BEL[2], mg/day	162[x]	161[xy]	157[y]	4.00	0.056	158	161	160	159	163	4.30	0.233	0.713
ATTD of P, %	69.90[x]	70.26[xy]	67.98[y]	1.22	0.079	51.16	66.67	68.82	78.99	81.25	1.37	< 0.001	< 0.001
STTD of P, %	73.10[xy]	73.50	71.23	1.22	0.079	54.39	69.92	72.07	82.24	84.5	1.37	< 0.001	< 0.001
Ca Digestibility													
Ca intake, g/day	6.29[x]	6.19[xy]	6.03[y]	0.15	0.056	6.12	6.18	6.15	6.13	6.28	0.17	0.277	0.635
Ca in feces, %	1.07[b]	1.20[a]	1.28[a]	0.09	<0.001	1.70	1.13	1.15	0.91	1.02	0.10	< 0.001	< 0.001
Fecal Ca excretion, g/day	1.06[b]	1.17[a]	1.25[a]	0.07	0.005	1.72	1.17	1.13	0.81	0.96	0.08	< 0.001	< 0.001
Absorbed Ca, %	5.23[a]	5.03[a]	4.78[b]	0.12	<0.001	4.4	5.01	5.02	5.32	5.32	0.13	< 0.001	< 0.001

Table 4.6. (cont.)

ATTD of Ca, %	83.15[a]	81.18[ab]	79.20[b]	0.92	<0.001	71.92	81.11	81.3	86.86	84.75	1.06	< 0.001	< 0.001
STTD of Ca, %	89.07[a]	87.09[ab]	85.15[b]	0.92	<0.001	77.81	87.04	87.22	92.77	90.67	1.06	< 0.001	< 0.001
BEL[2], mg/day	372[x]	366[xy]	357[y]	9.12	0.056	360	366	364	362	372	9.80	0.233	0.713

[a-b] Means within a row lacking a common subscript letter are different ($P < 0.05$). [x-y] Means within a row lacking a common subscript letter tend to be different ($P < 0.10$).

[1] Interactions between source of soybean meal and level of phytase were not significant. Therefore, only main effects are presented. Data are least squares means; n = 40 for soybean meal sources and n = 24 for phytase levels.

[2]BEL = basal endogenous loss; BEL was calculated by multiplying daily DM intake of pigs by BEL of Ca or P.

[3]Values for the STTD of Ca were calculated by correcting the ATTD of Ca with the average BEL of Ca (i.e., 433 mg/kg DM intake, Lee and Stein, 2023); values for the STTD of P were calculated by correcting the ATTD of P with the average BEL of P (i.e., 190 mg/kg of DM intake; NRC, 2012).

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CHAPTER 5: Increasing levels of phytase in corn-soybean meal diets increases the standardized total tract digestibility of Ca and other minerals in soybean meal when fed to growing pigs

Abstract

The objective of this experiment was to test the hypothesis that graded levels of microbial phytase increase the digestibility of Ca in soybean meal (**SBM**) when fed to growing pigs regardless of the source of SBM. A total of 160 pigs (initial BW = 14.13 ± 2.4 kg) were used in a randomized complete block design with 20 diets and 8 replicate pigs per diet. Three diets were formulated based on corn and 1 of 3 sources of SBM, and 12 additional diets were formulated by supplementing each of each of these diet with 500, 1,000, 2,000, or 4,000 phytase units (**FTU**)/kg. To calculate the digestibility of Ca in calcium carbonate at each phytase level, 5 additional diets based on corn and potato protein concentrate were formulated with 0, 500, 1,000, 2,000, or 4,000 FTU/kg. Results from these diets allowed for calculation of the digestible Ca provided from limestone to the corn-SBM diets at each level of phytase and subsequent calculation of digestibility of Ca in the Ca that originated from SBM. Pigs were fed experimental diets for 12 days, including 4 days of total fecal collection. No interactions between SBM source and phytase were observed. Source of SBM affected ($P < 0.05$) Ca intake and absorbed Ca, but did not affect apparent total tract digestibility (**ATTD**) or standardized total tract digestibility (**STTD**) of Ca in diets. However, source of SBM affected ($P < 0.05$) ATTD and STTD of P and the digestibility of some microminerals. Increasing phytase reduced (linear, $P < 0.01$) fecal output and increased (linear, $P < 0.01$) ATTD of dry matter. Phytase did not affect Ca or P intake, but decreased (linear, $P < 0.001$) fecal Ca and P excretion and increased (quadratic, $P < 0.01$) absorbed Ca and P as well as ATTD and STTD of Ca and P. Both ATTD and STTD of Ca in SBM increased (quadratic, $P \leq 0.05$) as phytase increased from 0 to 4,000 FTU/kg. Phytase

also increased (linear, $P < 0.05$) ATTD of K and Na, and decreased (linear, $P < 0.05$) ATTD of Fe and Mn. In conclusion, source of SBM did not affect Ca digestibility, but microbial phytase increased the release of Ca from SBM, indicating that Ca addition to SBM containing diets needs to be reduced if microbial phytase is used to avoid over-supplementation with Ca in diets.

Key words: calcium, microbial phytase, phosphorus, soybean meal

Abbreviations: AEE, acid hydrolyzed ether extract; ATTD, apparent total tract digestibility; DM, dry matter; FTU, phytase units; SBM, soybean meal; STTD, standardized total tract digestibility.

Introduction

Calcium is an essential macro mineral needed for growth, development, bone remodeling, regulation of enzyme activity, cellular signaling, and metabolic regulation in swine (Gonzalez-Vega and Stein, 2014). The concentration of Ca in diets and ingredients is important because of the negative impacts of excess Ca on the digestibility of P and other nutrients, as well as on feed intake and growth performance (Stein et al., 2011; Merriman et al., 2017; Lagos et al., 2019). Ideally, the requirement for Ca should be expressed as standardized total tract digestible (**STTD**) Ca instead of total Ca (NRC, 2012), which necessitates generation of values for digestibility of Ca in feed ingredients (Lautrou et al., 2020; Lee and Kong, 2022; Lee et al., 2023a). In plant feed ingredients, Ca concentration is low compared with feed minerals; however, Ca in soybean meal (**SBM**) ranges from 0.25% to 0.75% (Sotak-Pepper et al., 2016; Lee et al., 2023a). Because SBM is included at high proportions in swine diets, Ca analysis of SBM is important to be able to supplement diets with adequate quantities of additional Ca to meet requirements without including excess Ca in diets.

Phytase is an exogenous enzyme that catalyzes the hydrolysis of P from phytate, which increases P digestibility and releases other nutrients, including Ca, from the phytate matrix (Humer et al., 2014, Hu et al., 2022, Nelson et al., 2022). Phytase supplementation can reduce formation of insoluble Ca–phytate complexes, thereby increasing the digestibility of Ca in diets fed to weanling or growing pigs (Dersjant-Li and Dusel, 2019; Nelson et al., 2022). Because of the large negative impact of excess Ca on growth performance of pigs, it is important that the release of Ca by phytase is taken into account in diet formulations (Merriman et al., 2017; Lagos et al., 2019; Lee et al., 2023a). Digestibility of Ca is increased by phytase to a similar or greater extent as the digestibility of P is improved (Almeida et al., 2013; Lee et al., 2023b), but there are no data to document the release of Ca from SBM by graded levels of microbial phytase. However, if it is known how much Ca is released by different levels of phytase, the inclusion of Ca from limestone can be optimized in SBM-based diets containing phytase, which may prevent including excess Ca in diets. Therefore, the objective of this experiment was to test the hypothesis that graded levels of microbial phytase will gradually increase the digestibility of Ca in SBM regardless of the source of SBM.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment before animal work was initiated. Pigs used in the experiment were the offspring of Line 800 boars and Camborough females (PIC, Hendersonville, TN, USA).

One hundred and sixty growing pigs with an average initial body weight of 14.13 ± 2.4 kg were used in the experiment. Three sources of SBM were procured from J&R distributing

Inc., Lake Norden, SD, USA; Archer Daniels Midland Company, Decatur, IL, USA; and United Animal Health, Sheridan, IN, USA (Table 5.1). The 3 sources of SBM were designated as SBM 01, SBM 02, and SBM 03, respectively. Three basal corn-SBM diets were formulated using each of the 3 sources of SBM. Twelve additional diets were formulated by supplementing each basal diet with 500, 1,000, 2,000, or 4,000 phytase units (**FTU**)/kg. To determine the digestibility of Ca in calcium carbonate at each level of phytase inclusion, 5 diets based on corn and potato protein concentrate containing 0, 500, 1,000, 2,000, or 4,000 FTU/kg of microbial phytase were also formulated. Thus, a total of 20 experimental diets were used (Table 5.2). Because the concentration of Ca differed among the 3 sources of SBM, calcium carbonate was added to the diets at different levels to meet the requirement for Ca for 11 to 25 kg growing pigs (NRC, 2012). Pigs were housed individually in metabolism crates (0.67 × 0.80 m) that were equipped with a slatted floor, a feeder, and a nipple drinker. A screen floor for total fecal collection was installed below the slatted floor, which allowed for collection of feces and orts.

Pigs were randomly allotted to the 20 diets using a randomized complete block design with 4 blocks of 40 pigs (2 pigs per diet in each block) for a total of 8 replicate pigs per diet (Kim and Lindemann, 2007). Feed was provided daily in the amount of 3.5 times the energy requirement for metabolizable energy based on the initial body weight of pigs (i.e., 197 kcal metabolizable energy/kg of body weight$^{0.60}$; NRC, 2012). Water was available at all times. The daily allotment of feed was divided into 2 equal meals and provided at 0800 and 1600 h. Diets were fed for 12 days. The initial 5 days were the adaptation period to the diets, which was followed by 4 days of collection of feces according to the marker-to-marker approach (Adeola, 2001). In short on day 6, a color marker (i.e., indigo carmine) was added to the morning meal to mark the beginning of fecal collection, and the stop marker (i.e., ferric oxide) was added to the

morning meal on day 10 to mark the conclusion of fecal collection. Fecal samples were stored at -20°C immediately after collection. Orts collected during the collection period were dried at 65°C in a forced air oven (Heratherm OMH750; Thermo Fisher 1873 Scientific Inc., Waltham, MA, USA) and the dried weight was subtracted from the total provision of feed to each pig to calculate actual feed intake (Adeola, 2001). At the conclusion of the experiment, fecal samples were thawed and dried as explained for the orts, and ground through a 0.5mm screen using a grain mill (model: RRH-500, Zhejiang Winki Plastic Industry Co., Ltd., Zhejiang, China).

Chemical analysis Corn, potato protein concentrate, and SBM were analyzed for phytate (Ellis et al., 1977), and phytate in diets was calculated based on the analyzed phytate in each ingredient and the inclusion of corn, potato protein concentrate, or SBM in each diet. Phytase activity in diets was analyzed according to method 2000.12 (AOAC Int., 2019). Diets, corn, potato protein concentrate, SBM, and feces were analyzed for gross energy using bomb calorimetry (Model 6400, Parr Instruments, Moline, IL, USA). Samples were also analyzed for dry matter (**DM;** method 930.15; AOAC Int., 2019) and ash (method 942.05; AOAC Int., 2019). Concentrations of Ca, P, K, Mg, Na, Fe, Cu, Mn, and Zn in corn, potato protein concentrate, SBM, limestone, monosodium phosphate, diets, and dried fecal samples were analyzed (method 985.01 A, B and C; AOAC Int., 2019; Table 5.4) using inductively coupled plasma-optical emission spectrometry (Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600 °C for 4 h according to method 942.05 (AOAC Int., 2019) and wet digestion with nitric acid according to method 3050 B (U.S.-EPA, 2000). Crude protein in corn, potato protein concentrate, SBM, and diets was calculated as analyzed nitrogen × 6.25, and nitrogen in all samples was analyzed by combustion (method 990.03; AOAC Int., 2019) using a LECO FP828 Nitrogen Analyzer (LECO Corp., Saint

Joseph, MI, USA). Diets and ingredients were also analyzed for acid hydrolyzed ether extract (method 2003.06; AOAC Int., 2019; Ankom XT15 Extractor; Ankom Technology, Macedon, NY).

Calculations and statistical analysis

Phytate-bound P in corn, potato protein concentrate, SBM, and each diet was calculated by multiplying the analyzed phytate by 0.282 (Tran and Sauvant, 2004), and non-phytate P was calculated by subtracting phytate-bound P from total P. Feed intake was calculated by subtracting the weight of dried orts from feed provisions.

The apparent total tract digestibility (**ATTD**) of Ca and P in all diets was calculated using the following equation (Almeida and Stein, 2010):

$$\text{ATTD, \%} = [\text{intake - output / intake}] \times 100$$

where ATTD is in % and intake and output of Ca or P in of feces are expressed as gram per day. The ATTD of DM and minerals other than Ca and P were calculated as explained for Ca and P. Values for standardized total tract digestibility (**STTD**) of Ca and P were calculated using the following equation (Almeida and Stein, 2010):

$$\text{STTD, \%} = [(\text{intake - (output - daily basal endogenous loss)} / \text{intake}] \times 100$$

The basal endogenous loss of Ca was assumed to be 433 mg/kg of DM intake (Lee and Stein, 2023), and the basal endogenous loss of P was assumed to be 190 mg/kg of DM intake (NRC, 2012). The ATTD and STTD of Ca in calcium carbonate and the impact of each level of phytase on Ca digestibility was calculated from the 5 diets containing potato protein concentrate. The contribution of Ca from calcium carbonate to each of 15 diets containing SBM was then calculated and the ATTD and STTD of Ca in each source of SBM and within each level of phytase were calculated using the difference procedure (Adeola, 2001).

Data were analyzed using mixed procedures in SAS (SAS Inst. Inc., Cary, NC, USA) with the pig as the experimental unit. Outliers were determined by plotting the residuals in a quantile-quantile plot against the normal distribution and identifying values that were beyond ± 2.0 standard deviations. Normality and homogeneity of data were verified using the UNIVARIATE and MIXED procedures in SAS. Treatment means were calculated using the LSMEANS statement in SAS. The initial model included SBM source, phytase, and the interaction between SBM source and phytase as fixed variables and block and replicate within block as random variables. However, no interactions between SBM source and phytase were observed, and the final model, therefore, contained only source of SBM and phytase. Contrast statements were used to determine linear and quadratic effects of increasing phytase in diets. Unequally spaced contrasts were calculated using the PROC IML procedure of SAS. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Pigs remained healthy during the experiment and consumed their assigned diets without apparent health problems. However, one pig fed the potato protein concentrate diet and 500 units of phytase died for unclear reasons during the adaptation period, but all other pigs completed the experiment. Analyzed nutrient composition in diets was in agreement with formulated values and the analyzed phytase in diets was also as calculated (Tables 5.3 and 5.4).

Feed intake, day fecal output, and ATTD of DM were not affected by SBM source (Table 5.5). Calcium intake differed among SBM sources ($P \leq 0.001$), with greater Ca intake in diets containing SBM 01 compared with SBM 02. Absorbed Ca was also greater ($P < 0.05$) for

pigs fed SBM 01 compared with SBM 02 or SBM 03, but ATTD and STTD of Ca in diets did not differ among SBM sources.

Increasing phytase supplementation in diets reduced (linear, $P < 0.01$) the dry feces output and increased (linear, $P < 0.01$) the ATTD of DM, and the ATTD of GE tended to increase (linear $P < 0.10$) as phytase inclusion was increased in diets fed to pigs. Intake of Ca was not affected by phytase, but Ca in feces and fecal excretion of Ca decreased (quadratic, $P < 0.001$) as phytase supplementation in diets increased. Therefore, absorbed Ca, and ATTD and STTD of Ca also increased (quadratic, $P < 0.001$) as phytase levels increased.

The intake of P was not different among SBM sources. Concentration of P was greater ($P < 0.05$) in feces from pigs fed SBM 02 than in feces from pigs fed SBM 03 and fecal excretion of P was greater ($P < 0.05$) for pigs fed SBM 02 compared with SBM 01; whereas, absorbed P was greater ($P < 0.05$) for pigs fed diets containing SBM 01 compared with SBM 02. The ATTD and STTD of P were also greater ($P < 0.05$) for pigs fed SBM 01 than for pigs fed SBM 02, but the daily basal endogenous loss was not different among SBM sources.

Phytase supplementation did not affect P intake, but increasing phytase in diets reduced (quadratic, $P < 0.001$) the concentration of P in the feces and daily fecal P excretion. Phytase increased (quadratic, $P < 0.001$) absorbed P. The ATTD and STTD of P were also increased (quadratic, $P < 0.001$) by increasing phytase in diets, whereas basal endogenous loss of P was not affected by dietary phytase. The ATTD and STTD of Ca in SBM did not differ among SBM sources, but ATTD and STTD of Ca increased with phytase (linear, $P < 0.001$; quadratic, $P \leq 0.05$).

The K intake and absorbed K were greater ($P < 0.05$) for pigs fed SBM 01 or SBM 02 compared with pigs fed SBM 03, but ATTD of K did not differ among SBM sources (Table 5.6).

Increasing phytase reduced (linear, $P < 0.001$) K excretion and increased (linear, $P < 0.05$) absorbed K. Likewise ATTD of K increased (linear, $P < 0.001$) with increasing phytase in diets. The intake and fecal excretion of Mg were greater ($P < 0.01$) for pigs fed SBM 02 than for pigs fed SBM 01 and SBM 03, but SBM source did not affect ATTD of Mg. Increasing phytase did not affect Mg intake, absorbed Mg or ATTD of Mg.

For Na, pigs fed SBM 03 had greater ($P \leq 0.05$) Na intake and fecal Na excretion compared with SBM 02 or SBM 03. Absorbed Na tended ($P < 0.10$) to be greater for pigs fed SBM 03 compared with pigs fed SBM 01, but ATTD of Na was not different among SBM sources. Phytase reduced fecal Na excretion (linear, $P < 0.05$) and tended (linear, $P = 0.051$) to increase absorbed Na. Likewise, phytase supplementation increased (linear, $P < 0.01$) the ATTD of Na.

Intake of Cu was greater ($P < 0.05$) for pigs fed SBM 03 than the other sources of SBM, whereas pigs fed SBM source 02 had the least ($P < 0.05$) intake of Cu (Table 5.7). Fecal excretion of Cu was also greater ($P < 0.05$) from pigs fed SBM 03 than from pigs fed SBM 01 or SBM 02, and absorbed Cu was greater ($P < 0.05$) for pigs fed SBM 03 than SBM 01 or SBM 02, whereas absorbed Cu was greater ($P < 0.05$) for SBM 01 than for SBM 02. However, ATTD of Cu was less ($P < 0.05$) in SBM 02 than in SBM 01 and SBM 03. Increasing phytase did not affect Cu intake, excretion of Cu or ATTD of Cu but absorbed Cu was increased by phytase (quadratic, $P < 0.01$).

Intake, fecal excretion, and absorbed Fe were greater ($P < 0.001$) for SBM 03 than for SBM 01 or SBM 02, but ATTD of Fe was not impacted by source of SBM. Increasing phytase tended to increase (linear $P < 0.10$) excreted Fe and reduced (linear $P < 0.05$) absorbed Fe and ATTD of Fe. Intake of Mn and ATTD of Mn were greater ($P < 0.05$) for pigs fed diets

containing SBM 03 than SBM 02 or SBM 01. Increasing phytase in diets tended (linear, $P < 0.10$) to reduce absorbed Mn and ATTD of Mn.

Intake of Zn was greater ($P < 0.05$) for pigs fed SBM 03 than SBM 01 or SBM 02, but intake was less ($P < 0.05$) for SBM 01 than for SBM 02. Fecal excretion of Zn, absorbed Zn and ATTD of Zn, were also greater ($P < 0.05$) for SBM 03 than for SBM 01 and SBM 02. However, there were no impact of microbial phytase on intake, excretion, absorption, or ATTD of Zn.

Discussion

Analyzed concentrations of Ca and P in corn, potato protein concentrate, SBM, limestone, and monosodium phosphate were in agreement with published values (NRC, 2012). Analyzed phytase for some treatments was greater than expected which likely is due to overage in the phytase concentrate that was used. Phytate concentration in SBM ranged from 1.42 to 1.51%, which is slightly less than previously reported (Tahir et al., 2012; Lee et al., 2023a). Calcium in the 3 sources of SBM ranged from 0.20 to 0.57%, which is within the range of reported values (Sotak- Peper et al., 2015; Lee et al., 2023a).

The observation that there were no interactions between source of SBM and phytase indicates that all sources had a similar response to phytase, which is in agreement with previous data (Sotak-Peper et al., 2016). The differences in intake of Ca among the 3 sources of SBM were mainly a consequence of differences in feed intake and dietary Ca concentration rather than differences in the proportion of Ca that was digested. This observation agrees with data concluding that there are no differences in ATTD of Ca among soybean meals from different U.S. production areas (Sotak-Peper et al., 2016). It is likely that the reason SBM 02 and SBM 03 containing more Ca than SBM 01 is that the producers of these SBM added limestone to the

SBM to increase flowability because there is no difference in Ca among sources of full fat soybean (Ruiz et al., 2025). The addition of limestone to SBM 02 and SBM 03 likely also contributed to the lack of differences in ATTD and STTD of Ca. In contrast, the differences in ATTD and STTD of P that were observed among the 3 sources of SBM indicate that variation among SBM sources influence P digestibility more than Ca digestibility. Previous work with SBM also demonstrated that phytase increases P digestibility, but most of those experiments were conducted to estimate P release by phytase rather than the Ca contribution from phytase (Rojas and Stein, 2012; Sotak-Peper et al., 2016).

The observation that supplementation of phytase to diets reduced dry fecal output and increased ATTD of DM and GE, is in agreement with data demonstrating increased DM digestibility in diets containing increasing levels of phytase (Arredondo et al., 2019). Likewise, the increased ATTD and STTD of Ca and P that were calculated as phytase increased in diets demonstrates that microbial phytase improves Ca and P digestibility in SBM or corn–SBM diets fed to pigs as has been reported multiple times before (Rojas and Stein, 2012; Sotak-Peper et al., 2016; She et al., 2017; 2018; Arredondo et al., 2019). The observation that microbial phytase increased the ATTD and STTD of Ca in SBM supports the hypothesis that some Ca in SBM is bound to phytate, but the bond between inositol and P can be hydrolyzed by the phytase enzyme and thereby also release the Ca that was bound to the P in the phytate complex. We are not aware of any previous data demonstrating this effect for STTD of Ca in SBM. The increase in calculated Ca digestibility of SBM as phytase increased from 0 to 4,000 FTU/kg (from 64% to 91%) indicates that increased values for STTD of Ca in SBM needs to be used if phytase is included in SBM-containing diets. Using an increased STTD of Ca in diets with phytase will contribute to reducing the risk of providing excess of Ca in diets for pigs.

The increased ATTD of K and Na, that was observed as dietary phytase increased agrees with previous data (Zeng et al., 2014; Arredondo et al., 2019; Lagos et al., 2022). However, the lack of an increase in the ATTD of Mg as a result of phytase is in contrast with previous data (Zeng et al., 2014; She et al., 2018; Arredondo et al., 2019; Lagos et al., 2022). The lack of a Mg response in the present work may be a result of the ATTD of Mg in all diets being greater than the average ATTD of Mg observed in previous experiments (i.e., around 25%; Stein, 2024).

The observation that there were no differences among the 3 sources of SBM in the ATTD of K, Na, and Mg, demonstrates that source of SBM does not impact the digestibility of these minerals regardless of the level of microbial phytase in diets. However, the Mg in the diets was a combination of Mg from corn and SBM, and the Na in the diets originated from corn, SBM, and NaCl. The ATTD of Mg and Na, therefore, reflects the ATTD of combined sources.

Trace mineral responses to microbial phytase were more variable than macro mineral responses. Effects of SBM source were observed for ATTD of Cu, Mn, and Zn, but phytase did not affect ATTD of Cu or Zn and decreased ATTD of Fe and Mn. These results are not completely in agreement with previous reports. Arredondo et al. (2019) observed an increase in ATTD of Zn but no response for Cu, Fe, or Mn; whereas, Zeng et al. (2014) reported linear increases in Zn digestibility and a quadratic increase in Cu digestibility as phytase was increased in diets; She et al. (2018) reported increased ATTD of Fe; and Adeola et al. (1995) reported improved Cu and Zn utilization in pigs fed phytase. Therefore, the lack of a Zn response and the reductions in ATTD of Fe and Mn observed in the present experiment were unexpected. However, the premix used in the experiment contained Cu as basic copper carbonate and Zn as zinc carbonate; whereas, Cu sulfate and Zn oxide were used in previous experiments. It is possible that differences in solubility among the added sources of Cu and Zn altered interactions

with phytate and Ca and thereby contributed to the responses observed in this experiment. However, more researcher is needed to confirm this hypothesis. Some resent data have shown that in weaned pigs, the use of 1,500 FTU/kg of microbial phytase replaced the added trace Zn, Cu, Fe and Mn, however, the levels that we found in our diets were high than the positive control diets using in the experiment (Marchal et al., 2026).

Conclusions

The hypothesis that ATTD and STTD of Ca in SBM was increased as microbial phytase was included in diets was accepted and it is, therefore, important to use a greater value for ATTD of Ca in SBM in such diets. The lack of a difference among the 3 sources of SBM, despite differences in Ca concentration, indicates that the demonstrated increases in STTD of Ca likely is uniform among sources of SBM. Increases in ATTD of most other macro minerals were also observed as microbial phytase inclusion increased in diets.

Disclosures

The authors have no conflicts of interest.

Tables

Table 5.1. Analyzed composition of feed ingredients, as-fed basis

Item	Soybean meal			Corn	Potato protein concentrate	Calcium carbonate	Monosodium phosphate
	01	02	03				
Dry matter, %	88.45	88.32	88.82	87.95	91.26	99.7	99.1
Gross energy, kcal/kg	4,136	4,119	4,135	3,918	5,334	-	-
Crude protein, %	47.37	45.08	46.5	7.50	80.34	-	-
Acid ether extract, %	2.41	2.14	2.75	2.70	2.82	-	-
Ash, %	5.71	6.13	6.43	1.30	0.13	91.53	89.20
Phytate, %	1.51	1.50	1.42	0.79	< 0.14	-	-
Ca, %	0.25	0.40	0.57	0.02	0.01	39.13	0.01
P, %	0.69	0.69	0.67	0.27	0.02	0.24	26.75
Phytate-bound P[1]	0.43	0.42	0.4	0.22	-	-	-
Non-phytate-P[2]	0.26	0.27	0.27	0.05	0.02	-	-
Mg, %	0.26	0.29	0.26	0.35	<0.01	0.64	0.72
K, %	2.06	1.98	1.81	0.01	0.06	0.30	0.10
Na, %	0.04	0.07	0.08	0.10	<0.01	0.04	0.01
Cu, mg/kg	24.70	19.40	79.30	122.50	32.00	-	8.91
Fe, mg/kg	164.10	113.90	503.30	3.00	328.00	776.00	0.61
Mn, mg/kg	55.80	43.90	193.70	18.70	3.00	63.60	0.05
Zn, mg/kg	72.20	67.60	566.40	4.90	21.00	-	121.20

[1]Phytate-P was calculated as 28.2% of total phytate (Tran and Sauvant, 2004).

[2]Non-phytate P was calculated by subtracting phytate bound P from total P.

Table 5.2. Ingredient composition and expected energy and nutrient composition of experimental diets[1], as-fed basis

Item, %	Soybean meal 01	Soybean meal 02	Soybean meal 03	Potato protein
Ground corn	55.31	55.56	55.70	77.31
Soybean meal 01	40.00	-	-	-
Soybean meal 02	-	40.00	-	-
Soybean meal 03	-	-	40.00	-
Potato protein	-	-	-	18.00
Soybean oil	1.80	1.80	1.80	1.00
Calcium carbonate	1.11	0.86	0.76	1.37
Monosodium phosphate	0.25	0.25	0.25	0.68
L-Lysine HCL, 78% Lys	0.09	0.09	0.09	0.24
DL-Methionine, 98% Met	0.04	0.04	0..04	-
Phytase premix[2]	0.50	0.50	0.50	0.50
Vitamin-mineral premix[3]	0.50	0.50	0.50	0.50
Salt	0.40	0.40	0.40	0.40

[1]For each source of soybean meal there was one diet without phytase and 4 diets containing 500, 1,000, 2,000, or 4,000 units of phytase. There were also five diets with potato protein concentrate, and 0, 500, 1,000, 2,000, or 4,000 units of phytase

[2]Five separate premixes were prepared to provide 0, 500, 1,000, 2,000, or 4,000 units of phytase in the final diets by mixing ground corn (100, 98, 96, 92, or 84%) and 0, 2, 4, 8, or 16% of the phytase concentrate (Quantum Blue, AB Vista, Marlborough, UK; 5,000 phytase units per g).

[3]The vitamin–mineral premix provided the following quantities of vitamins and micro-minerals

Table 5.2. (cont.)

per kilogram of complete diet: vitamin A as retinyl acetate, 7,992 IU; vitamin D_3 as cholecalciferol, 1,598 IU; vitamin E as dl-α-tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfite, 1.50 mg; vitamin B_{12}, 0.03 mg; riboflavin, 6.00 mg; D-pantothenic acid as D-calcium pantothenate, 24.0 mg; niacin, 44.0 mg; biotin, 0.43 mg; folic acid, 2.0 mg; pyridoxine as pyridoxine hydrochloride, 8.0 mg; thiamine as thiamine mononitrate, 1.5 mg; Cu as basic copper carbonate, 20 mg; Fe as ferrous sulfate, 62.5 mg; I as ethylenediamine dihydroiodide, 0.50 mg; Mn as manganous oxide, 30 mg; Se as sodium selenite, 0.30 mg; and Zn as zinc carbonate, 125 mg per kilogram of complete diet.

[4] Values calculated from analyzed ingredients, NRC (2012), and Lee et al. (2023a).

[5]STTD, standardized total tract digestibility P (NRC, 2012).

[6]Phytate in all diets was calculated based on analyzed phytate in corn, potato protein concentrate, and soybean meal. Phytate-bound P was calculated by multiplying the analyzed phytate by 0.282 (Tran and Sauvant, 2004). [2]Non-phytate P was calculated by subtracting phytate bound P from total P.

Table 5.3. Analyzed nutrient composition of diets, as-fed basis

Item,	Dry matter, %	Gross energy, kcal/kg	Crude protein, %	Ash, %	AEE[1], %	Ca, %	Non-phytate P[2], %	FTU[1]/kg
0 phytase unit/kg								
Soybean meal 01	88.42	4,021	23.50	4.55	4.13	0.52	0.26	79
Soybean meal 02	88.50	4,022	22.45	4.57	4.84	0.55	0.27	<70
Soybean meal 03	88.11	3,984	22.50	4.77	4.55	0.61	0.26	88
Potato protein concentrate	88.55	4057	21.50	2.97	3.3	0.61	0.17	<70
500 phytase unit/kg								
Soybean meal 01	88.18	4,008	23.00	4.54	4.68	0.51	0.26	670
Soybean meal 02	88.22	3,986	22.85	4.63	4.09	0.54	0.27	480
Soybean meal 03	88.72	4,055	23.35	4.93	4.40	0.60	0.26	680
1,000 phytase unit/kg								
Potato protein concentrate	88.61	4,054	21.00	2.87	3.25	0.60	0.17	620
Soybean meal 01	88.31	4,034	23.60	4.42	4.49	0.51	0.26	1,100
Soybean meal 02	88.22	4,009	22.25	4.53	4.14	0.53	0.27	1,200
Soybean meal 03	88.19	3,994	22.60	4.72	4.27	0.61	0.26	1,300

Table 5.3 (cont.)

2,000 phytase unit/kg								
Potato protein concentrate	88.07	4,036	20.75	2.96	2.96	0.60	0.17	1,200
Soybean meal 01	88.05	4,016	24.15	4.47	3.74	0.50	0.26	2,600
Soybean meal 02	87.7	3,982	22.55	4.53	4.48	0.54	0.27	2,000
Soybean meal 03	88.12	3,971	22.80	4.59	4.50	0.60	0.26	2,300
Potato protein concentrate	88.57	4,063	21.60	2.97	3.05	0.61	0.17	2,800
4,000 phytase unit/kg								
Soybean meal 01	88.18	4,033	23.95	4.44	3.41	0.53	0.26	4,500
Soybean meal 02	87.64	3,978	22.90	4.48	3.68	0.54	0.27	4,800
Soybean meal 03	87.61	3,971	22.40	4.74	3.38	0.61	0.26	4,600
Potato protein concentrate	88.15	4,054	21.50	3.03	3.28	0.60	0.17	4,500

[1]AEE = acid hydrolyzed ether extract; FTU = units of microbial phytase.

Table 5.4. Analyzed mineral concentration of diets, as-fed basis

Item	K, %	Mg, %	Na, %	Cu, mg/kg	Fe, mg/kg	Mn, mg/kg	Zn, mg/kg
0 phytase units/kg							
Soybean meal 01	0.91	0.15	0.20	27.93	182.61	76.59	149.77
Soybean meal 02	0.85	0.16	0.23	29.61	190.68	84.85	179.82
Soybean meal 03	0.89	0.15	0.24	47.71	336.48	136.10	358.61
Potato protein	0.24	0.07	0.29	29.77	155.16	62.50	127.81
500 phytase units/kg							
Soybean meal 01	0.91	0.16	0.25	30.30	192.78	73.06	151.26
Soybean meal 02	0.97	0.16	0.25	30.82	190.40	73.77	153.66
Soybean meal 03	0.88	0.15	0.27	54.52	366.85	153.57	403.68
Potato protein	0.23	0.07	0.26	30.31	147.21	60.13	122.67
1,000 phytase units/kg							
Soybean meal 01	0.94	0.14	0.24	26.19	167.97	72.49	139.39
Soybean meal 02	0.98	0.15	0.26	31.21	184.86	79.57	172.07
Soybean meal 03	0.89	0.08	0.26	49.37	345.01	149.01	378.66
Potato protein	0.26	0.06	0.28	26.01	147.32	58.36	123.68
2,000 phytase units/kg							
Soybean meal 01	0.95	0.15	0.26	27.43	159.87	69.77	129.56
Soybean meal 02	1.00	0.16	0.24	23.59	171.02	73.43	153.61
Soybean meal 03	0.90	0.14	0.24	51.23	341.70	148.55	371.94
Potato protein	0.28	0.07	0.28	33.47	158.32	62.71	133.94

Table 5.4 (cont.)

4,000 phytase units/kg							
Soybean meal 01	0.95	0.14	0.21	28.36	166.87	71.32	134.36
Soybean meal 02	1.04	0.16	0.23	27.15	172.39	76.22	146.11
Soybean meal 03	0.95	0.14	0.27	56.00	385.25	164.86	433.43
Potato protein	0.28	0.07	0.28	30.21	158.69	61.17	125.55

Table 5.5 Apparent total tract digestibility (ATTD) and standardized digestibility (STTD) of Ca and P in diets containing corn and 1 of 3 sources of soybean meal (SBM) and graded levels of phytase and ATTD and STTD of Ca in each source of SBM[1,2].

Item	Soybean meal, source			SEM	*P*-value	Phytase, phytase unit/kg					SEM	*P*-value	
	01	02	03		SBM	0	500	1,000	2,000	4,000		Linear	Quadratic
Feed intake, g/day	917	908	803	16.07	0.728	904	889	941	915	898	19.30	0.822	0.193
Dry feces output, g/d	92.2	96.9	95.3	3.61	0.433	100.0	91.7	101.8	93.5	86.9	4.18	0.008	0.557
ATTD of DM, %	89.5	88.9	88.9	0.43	0.200	88.4	89.3	88.7	89.2	89.8	0.48	0.006	0.921
ATTD of GE, %	87.6	86.8	87.2	0.489	0.203	86.8	87.5	86.7	87.3	87.8	0.548	0.093	0.811
Ca digestibility													
Ca intake, g/day	4.88[a]	4.52[b]	4.73[ab]	0.08	0.001	4.68	4.60	4.88	4.74	4.65	0.10	0.841	0.179
Ca in feces, %	1.24	1.19	1.29	0.04	0.258	1.69	1.22	1.18	1.08	1.03	0.05	< 0.001	< 0.001
Fecal Ca excretion, g/day	1.15	1.15	1.23	0.06	0.359	1.68	1.13	1.18	1.01	0.90	0.07	< 0.001	< 0.001
Absorbed Ca, g/day	3.73[a]	3.37[b]	3.50[b]	0.09	< 0.001	3.00	3.47	3.69	3.74	3.76	0.10	< 0.001	< 0.001
ATTD of Ca, %	76.3	74.6	74.0	1.31	0.182	64.00	75.8	75.6	79.0	80.7	1.50	< 0.001	< 0.001
STTD of Ca, %	83.5	82.4	81.4	1.31	0.245	71.3	83.3	83.1	86.4	88.1	1.50	< 0.001	< 0.001

Table 5.5 (cont.)

P intake, g/day	4.51	4.46	4.38	0.08	0.332	4.42	4.35	4.60	4.48	4.40	0.09	0.828	0.196
P in feces, %	1.44[ab]	1.56[a]	1.42[b]	0.05	0.045	2.14	1.53	1.43	1.22	1.06	0.06	< 0.001	< 0.001
Fecal P excretion, g/day	1.35[b]	1.51[a]	1.36[ab]	0.09	0.022	2.13	1.41	1.43	1.14	0.92	0.09	< 0.001	< 0.001
Absorbed P, %	3.16[a]	2.95[b]	3.02[ab]	0.10	0.046	2.30	2.94	3.17	3.34	3.48	0.11	< 0.001	< 0.001
Basal EPL, mg/day	154	153	153	2.71	0.826	152	150	159	154	152	3.26	0.863	0.125
ATTD of P, %	70.1[a]	66.2[b]	68.9[ab]	1.94	0.012	51.9	67.9	68.6	74.6	79.0	2.09	< 0.001	< 0.001
STTD of P, %	73.5[a]	69.7[b]	72.4[ab]	1.94	0.012	55.3	71.4	72.1	78.0	82.5	2.09	< 0.001	< 0.001
Soybean meal, Ca digestibility													
ATTD of Ca, %	77.9	71.8	71.3	5.5	0.356	55.7	68.8	77.4	80.4	86.0	6.19	< 0.001	0.023
STTD of Ca, %	83.6	79.9	78.4	5.5	0.569	63.7	76.7	82.5	89.3	90.9	6.18	< 0.001	0.014

[a-b] Means within a row lacking a common subscript letter are different ($P < 0.05$).

[1] Interactions between source of soybean meal and level of phytase were not significant. Therefore, only main effects are presented.

[2]Data are least squares means; n = 40 for soybean meal sources and n = 24 for phytase levels.

Table 5.6. Apparent total tract digestibility (ATTD) of macro minerals: K, Mg, and Na, in diets containing corn and 1 of 3 sources of soybean meal (SBM) graded levels of phytase in each source of SBM when fed to growing pigs.[1,2]

Item,	Soybean meal, source				*P*-value	Phytase, phytase unit/kg						*P*-value	
	01	02	03	SEM	SBM	0	500	1,000	2,000	4,000	SEM	Linear	Quadratic
K intake, g/day	8.57[a]	8.81[a]	8.12[b]	0.15	0.001	8.45	8.31	8.79	8.54	8.40	0.18	0.805	0.208
Fecal K excretion, g/day	1.07	1.16	1.10	0.08	0.231	1.31	1.08	1.21	1.04	0.89	0.09	< 0.001	0.527
Absorbed K, g/day	7.50[a]	7.65[a]	7.02[b]	0.17	0.001	7.15	7.23	7.57	7.50	7.51	0.20	0.010	0.132
ATTD of K, %	87.45	86.88	86.45	0.99	0.230	84.36	87.01	86.14	87.77	89.29	1.04	< 0.001	0.174
Mg intake, g/day	1.36[b]	1.45[a]	1.30[b]	0.02	< 0.001	1.36	1.34	1.41	1.38	1.35	0.03	0.775	0.193
Fecal Mg excretion, g/day	0.71[b]	0.79[a]	0.69[b]	0.03	0.003	0.78	0.68	0.77	0.71	0.71	0.04	0.322	0.572
Absorbed Mg, g/day	0.65	0.66	0.60	0.03	0.150	0.58	0.66	0.65	0.66	0.64	0.04	0.437	0.083
ATTD of Mg, %	48.05	45.19	46.45	2.11	0.398	43.06	49.70	45.53	48.20	46.67	2.43	0.538	0.196
Na intake, g/day	2.11[b]	2.19[b]	2.31[a]	0.04	< 0.001	2.19	2.15	2.28	2.22	2.18	0.05	0.827	0.181
Fecal Na excretion, g/day	0.27[b]	0.28[b]	0.34[a]	0.04	0.035	0.32	0.32	0.35	0.28	0.20	0.04	0.001	0.241
Absorbed Na, g/day	1.85[y]	1.92[xy]	1.97[x]	0.59	0.059	1.88	1.83	1.93	1.94	1.97	0.07	0.051	0.672
ATTD of Na, %	87.36	87.41	85.14	1.85	0.198	85.44	85.33	84.58	87.34	90.48	2.03	0.001	0.404

[a-b] Means within a row lacking a common subscript letter are different ($P < 0.05$).

[x-y] Means within a row lacking a common subscript letter tend to be different ($P < 0.10$).

Table 5.6. (cont.)

[1] Interactions between source of soybean meal and level of phytase were not significant. Therefore, only main effects are presented.

[2] Data are least squares means; n = 40 for soybean meal sources and n = 24 for phytase levels.

Table 5.7. Apparent total tract digestibility (ATTD) of micro minerals: Cu, Fe, Mn and Zn in diets containing corn and 1 of 3 sources of soybean meal (SBM) graded levels of phytase in each source of SBM when fed to growing pigs[1,2].

Item	Soybean meal, source			SEM	*P*-value	Phytase, phytase unit/kg					SEM	*P*-value	
	01	02	03		SEM	0	500	1,000	2,000	4,000		Linear	Quadratic
Cu intake, mg/day	287[b]	259[c]	467[a]	6.46	< 0.001	335	329	349	342	333	7.69	0.914	0.127
Fecal Cu excretion, mg/day	165[b]	174[b]	275[a]	11.85	< 0.001	209	188	212	209	206	13.27	0.685	0.788
Absorbed Cu, mg/day	120[b]	85.14[c]	192[a]	10.12	< 0.001	155	154	122	105	127	12.60	0.040	0.008
ATTD of Cu, %	42.21[a]	32.85[b]	41.26[a]	2.81	0.002	36.90	42.75	38.75	38.86	36.61	3.25	0.424	0.483
Fe intake, mg/day	1,596[b]	1,652[b]	3,205[a]	42.07	< 0.001	2,135	2,097	2,222	2,179	2,122	50.00	0.907	0.134
Fecal Fe excretion, mg/day	1,021[b]	1,082[b]	1,926[a]	68.40	< 0.001	1,311	1,197	1,395	1,378	1,436	80.50	0.052	0.710
Absorbed Fe, mg/day	575[b]	570[b]	1,279[a]	51.22	< 0.001	823	900	828	801	686	63.92	0.026	0.514
ATTD of Fe, %	36.24	34.55	40.06	2.48	0.166	37.96	42.40	36.60	37.20	30.58	3.01	0.009	0.487
Mn intake, mg/day	703[b]	705[b]	1,357[a]	17.96	< 0.001	915	899	953	934	909	21.36	0.910	0.131
Fecal Mn excretion, mg/day	468	490	821	30.05	< 0.001	588	530	619	604	625	35.00	0.117	0.783
Absorbed Mn, mg/day	235[b]	215[b]	537[b]	22.37	< 0.001	326	369	334	329	285	27.62	0.070	0.438
ATTD of Mn, %	33.65[ab]	30.53[b]	39.68[a]	2.61	0.008	34.52	40.25	33.81	35.25	29.29	3.106	0.034	0.420
Zn intake, mg/day	1,292[c]	1,461[b]	3,514[a]	43.60	< 0.001	2,073	2,037	2,157	2,121	2,058	51.52	0.929	0.133
Fecal Zn excretion, mg/day	1,088[b]	1,151[b]	2,344[a]	70.06	< 0.001	1,609	1,387	1,639	1,587	1,415	86.06	0.259	0.303
Absorbed Zn, mg/day	204[b]	312[b]	1,170[a]	67.31	< 0.001	463	650	518	535	645	79.89	0.223	0.827

Table 5.7 (cont.)

ATTD of Zn, %	15.91[b]	21.45[b]	33.30[a]	3.37	< 0.001	22.33	31.91	24.01	25.22	31.34	4.19	0.152	0.938

[a-b] Means within a row lacking a common subscript letter are different ($P < 0.05$). [x-y] Means within a row lacking a common subscript letter tend to be different ($P < 0.10$).

[1] Interactions between source of soybean meal and level of phytase were not significant. Therefore, only main effects are presented.

[2] Data are least squares means; n = 40 for soybean meal sources and n = 24 for phytase levels.

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Chapter 6: Effects of graded levels of phytase on standardized ileal digestibility of amino acids in soybean meal

Abstract

An experiment was conducted to test the hypothesis that increasing dietary concentrations of microbial phytase increases the apparent ileal digestibility (**AID**), and standardized ileal digestibility (**SID**) of crude protein (**CP**) and amino acids (**AA**) in soybean meal (**SBM**). The second hypothesis was that the source of SBM does not impact the response of growing pigs to microbial phytase. Three sources of SBM from different locations in the United States, were labeled SBM 01, SBM 02, and SBM 03, respectively. A total of 16 growing pigs (initial body weight 25 ± 3.6 kg) were equipped with a T-cannula in the distal ileal and allotted to 1 of 16 diets in a Youden Latin square design. Fifteen diets based on corn-starch and soybean meal were formulated by including 0, 500, 1,000, 2,000, or 4,000 phytase units/kg (**FTU**), to each source of SBM]; a nitrogen-free diet based on corn-starch was also formulated. Results indicate that AID and SID of CP and AA were not affected by SBM source, with only minor differences observed for Trp, Lys, and Gly. The AID of dispensable AA increased linearly ($P < 0.001$) with increasing inclusion of microbial phytase.. Increasing dietary phytase linearly improved ($P < 0.001$) both AID and SID of CP as well as all indispensable and dispensable AA. In conclusion, differences among SBM sources had minimal influence on the AID of CP and AA, but increasing concentrations of microbial phytase linearly improved both AID and SID of CP and all AA. Therefore, phytase improved protein and amino acid digestibility regardless of SBM source.

Key words: amino acid, apparent ileal digestibility, crude protein, standardized ileal digestibility, microbial phytase, soybean meal

Abbreviations: AA, amino acids, AEE, acid hydrolyzed ether extract; AID, apparent ileal digestibility; CP, crude protein; DM, dry matter; FTU, phytase units; SBM, soybean meal; SID, standardized ileal digestibility.

Introduction

Soybean meal (**SBM**) is a coproduct of soybean oil extraction, used in swine diets because of its high protein concentration and favorable amino acid (**AA**) profile (Stein et al., 2007; NRC, 2012, Lagos et al., 2017). The global market size of soybean meal is estimated at $106 billion, with production projected to reach approximately 290 million metric tons in 2025–2026 (USDA-FAS, 2026; The Business Research Company, 2026). The increasing demand for protein-rich animal feed is expected to drive continued growth of the soybean meal market (Gaffield et al., 2024; USDA-FAS, 2026; The Business Research Company, 2026). This growth highlights the importance of continued innovation, development, and research to better understand the nutritional value of SBM and to support its efficient use in diets for pig diets and other species (OECD and FAO, 2025; The Business Research Company, 2026).

Nevertheless, as feed costs remain high and environmental constraints continue to intensify, greater precision in diet formulation is needed to match the supply of digestible AA from key ingredients such as SBM with the requirements of pigs, thereby improving nutrient-use efficiency and reducing nitrogen excretion (van Milgen and Dourmad, 2015). However, the nutritional value of SBM is not uniform, and differences in soybean origin and processing conditions can affect ileal AA digestibility (Karr-Lilienthal et al., 2004; Gaffield et al., 2024). Therefore, the analysis and the accuracy of digestible AA values in SBM may provide both economic and environmental benefits (Bohlke et al., 2005; van Milgen and Dourmad, 2015;

Dersjant-Li and Dusel, 2019a). In swine nutrition, the use of phytase is common because it releases P from phytate, the main storage form of this mineral in oilseeds and grains. Beyond improving P release, phytase may also enhance the digestibility of AA in diets containing SBM (Moran et al., 2017; Dersjant-Li and Dusel, 2019a; Velayudhan et al., 2021, 2025).

Recently, combined data from 8 datasets generated from 5 experiments demonstrated improvements in ileal AA digestibility, using microbial phytase, with predicted increases of 3.7 and 4.5 % units in total AA digestibility at 1,000 and 4,000 units of phytase per kg, respectively (Velayudhan et al., 2025). Because Velayudhan et al. (2025) modeled mixed-diet datasets and described the outcome as diet-specific AA matrix recommendations, additional ingredient-focused data are needed to establish digestible AA matrix values specifically for SBM sources (Velayudhan et al., 2021, 2025).

Therefore, the objective of this experiment was to determine the ileal digestibility of AA in 3 sources of SBM supplemented with graded levels of microbial phytase. The first hypothesis was that increasing phytase inclusion would increase digestibility of AA in SBM. The second hypothesis was that the response to phytase was different among the 3 SBM sources.

Materials and methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment before animal work was initiated. Pigs used in the experiment were the offspring of Line 800 boars and Camborough females (PIC, Hendersonville, TN, USA).

Sixteen growing barrows were equipped with a T-cannula (barrel length: 6 cm; inner diameter: 1.6 cm) in the distal ileum. After 6 or 7 days of recovery, the pigs were weighted (initial body weight 25.65 ± 3.9 kg) and allotted to 1 of 16 diets in a 16 × 8 incomplete Latin square design with 16 pigs, 8 periods, and 1 pig per treatment in each period. Pigs were housed individually in 0.9 ×1.8 m pens that had fully slatted concrete floors and were equipped with a feeder and a cup waterer in an environmentally controlled room. The experimental animal allotment program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets.

Three sources of soybean meal (**SBM**) were procured from J&R distributing Inc., Lake Norden, SD, USA; Archer Daniels Midland Company, Decatur, IL, USA; and United Animal Health, Sheridan, IN, USA, respectively (Tables 6.1, and 6.2). The 3 sources were randomly labelled SBM 01, SBM 02, and SBM 03. Each source of SBM was included in 5 corn starch-SBM diets containing 0, 500, 1,000, 2,000, or 4,000 phytase units (**FTU)** per kg of diet (Quantum Blue, AB Vista, Marlborough, UK) and a nitrogen-free diet was also formulated (Table 6.3). All diets except the nitrogen-free diet were formulated to meet nutrient requirements for 25-50 kg pigs (NRC, 2012). The SBM included in each diet was the sole source of AA. All diets also contained 0.4% chromic oxide (Cl_2O_3) as an undigestible marker. A representative sample of 2 kg of each diet and ingredient was collected immediately after diet mixing and crude protein and phytase in all diets were analyzed before feeding of animals was initiated.
Feed was provided daily in the amount of 3.5 times the energy requirement for metabolizable energy based on the initial body weight of pigs (i.e., 197 kcal metabolizable energy/kg body

weight$^{0.60}$; NRC, 2012). Water was available at all times. The initial 5 days of each period were considered an adaptation period to the diet, and ileal digesta were collected for 9 h on days 6 and 7 of each period using standard operating procedures. In short, a plastic bag was attached to the cannula barrel and digesta flowing into the bag was collected. Bags were removed whenever they were filled with digesta - or at least once every 30 min and immediately stored at –20ºC to prevent bacterial degradation of AA in the digesta. On the completion of one experimental period, pigs were deprived of feed overnight and the following morning, a new experimental diet was offered. Ileal digesta samples were mixed and a sub-sample was collected.

Chemical analysis

Ileal digesta samples were thawed and a sub-sample was collected and lyophilized (Lagos and Stein, 2019). The 3 sources of SBM sources, limestone, dicalcium phosphate, and diet were ground in a grain mill model: capacity of 500 g, swing type grain mill (RRH, Rancho Cucamonga, CA, USA). Lyophilized ileal digesta samples were ground in a coffee grinder (model: 60 gr stainless steel blades, Mainstays, China). Phytase activity in diets was analyzed according to method 2000.12 (AOAC Int., 2019). Ingredients, diets and ileal digesta were analyzed for dry matter (**DM**; method 930.15; AOAC Int., 2019) and ingredients and diets were also analyzed for ash (method 942.05; AOAC Int., 2019). Diets, corn starch, and SBM were analyzed for gross energy using bomb calorimetry (Model 6400, Parr Instruments, Moline, IL, USA). The SBM, limestone, dicalcium phosphate, and diet were analyzed for Ca and P by inductively coupled plasma-optical emission spectrometry (method 985.01 A, B, and C; AOAC Int., 2019) after dry ash preparation (method 942.05; AOAC Int., 2019) followed by wet digestion with nitric acid (method 3050 B; US-EPA, 2000). Diets, SBM sources, and ileal digesta samples were analyzed for AA [method 982.30 E (a, b, c); AOAC Int., 2019] using a

Hitachi AA Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, USA). Prior to analysis, samples were hydrolyzed with 6 *N* HCl for 24 h at 110 °C [method 982.30 E(a); AOAC Int., 2019]. Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis [method 982.30 E(b); AOAC Int., 2019]. Tryptophan was determined after NaOH hydrolysis for 22 h at 110 °C [method 982.30 E(c); AOAC Int., 2019]. Diets and ileal digesta samples were also analyzed for Cr (Method 990.08; AOAC Int., 2019). Corn-starch, SBM sources, diet, and ileal digesta samples were also analyzed for nitrogen by the combustion procedure (method 990.03; AOAC Int., 2019) using a LECO FP828 (LECO Corp., Saint Joseph, MI, USA) and crude protein (**CP**) was calculated as nitrogen × 6.25. Corn-starch, SBM, and diets were analyzed for acid hydrolyzed ether extract (**AEE**; method 2003.06; AOAC Int., 2019; Ankom XT15 Extractor; Ankom Technology, Macedon, NY). The 3 SBM sources were also analyzed for trypsin inhibitors (method Ba 12-75; AOCS, 2006).

Calculations and statistical analysis

The apparent ileal digestibility (**AID**) and standardized ileal digestibility (**SID**) of CP and AA were calculated using the analyzed CP, AA, and Cr concentrations in the diet and ileal digesta samples (Stein et al., 2007). Basal endogenous losses of CP and AA were calculated from pigs fed the nitrogen-free diet as previously described (Stein et al., 2007). Values for AID and SID of CP and AA calculated for each diet also represented the AID and SID of CP and AA in the 3 sources of SBM, because SBM was the only source of AA in each diet. Concentrations of standardized ileal digestible AA were calculated by multiplying the analyzed CP and AA in each source of soybean by the digestibility value for CP and each AA.

Data were analyzed using mixed procedures in SAS (SAS Inst. Inc., Cary, NC, USA) with the pig being the experimental unit. Outliers were determined by plotting the residuals in a quantile-quantile plot against the normal distribution and identifying values that were beyond ± 2.0 standard deviations. Normality and homogeneity of data were verified using the UNIVARIATE and MIXED procedures in SAS. Data were analyzed by ANOVA using the PROC MIXED of SAS in a randomized complete block design with 16 × 8 Youden square design. Pig will be the experimental unit. The statistical model included diet as fixed variable and period and animal as random variables.

The initial model included SBM source, phytase, and the interaction between SBM source and phytase as fixed variables. Within each source of SBM and for the 3 sources combined, the effect of adding increasing levels of phytase on the SID of AA was determined using linear and quadratic contrast statements. Statistical significance and tendencies will be considered at $P < 0.05$, $P \leq 0.05$, and $P < 0.10$, respectively.

Results

Pigs remained healthy during the experiment and no pigs had to be removed from the experiment. Pigs easily consumed their diets and limited feed refusals were observed. Ingredients and diets were analyzed before the animal part of the experiment was initiated and correct diet mixing was confirmed (Table 6.4).

Apparent ileal digestibility of crude protein and amino acids

There was no effect of SBM source on the AID of CP or most AA (Table 6.5). However, AID of dry matter was greater ($P < 0.05$) on diets containing SBM 01 or SBM 03, compared

with SBM 02. The AID of Trp was greater ($P < 0.05$) for diets containing SBM 01 compared with SBM 02 or SBM 03.

Increasing phytase in the diets increased (linear, $P < 0.001$) the AID of CP and indispensable AA. Likewise, the AID of dispensable AA was increased ($P < 0.001$) as microbial phytase was increased in the diets.

Standardized ileal digestibility of crude protein and amino acids

The SID of CP was greater ($P \leq 0.05$) in ileal digesta samples from pigs fed SBM 03 than in ileal digesta from pigs fed SBM 02 (Table 6.6). Likewise, the SID of Lys and Gly was greater ($P < 0.05$) in SBM 03 than in SBM 02, and the SID of Trp was greater ($P < 0.05$) in SBM 01 than in SBM 02. However, no differences among SBM sources were observed for the SID of all other AA.

Increasing phytase in the diets increased (linear, $P < 0.001$) SID of CP and all indispensable and dispensable AA.

Discussion

The observation that the source of SBM had no effect on the SID of most AA agrees with previous work indicating that SBM from different sources or production regions in the U.S. has similar AA digestibility (Sotak-Peper et al., 2017; Lagos et al., 2017). The observation that the SID of Lys and Trp was different among the 3 sources of SBM likely is of minor importance because the differences, although significant, were small. Lysine is particularly susceptible to heat damage during processing, and overheating may reduce the SID of Lys more than for most other AA (Stein et al., 2007). It is, therefore, possible that the small differences in the SID of Lys that were observed were due to differences in temperatures used in toasting of SBM. However,

because there were no differences in SID of most other AA, it is likely that processing differences among the 3 SBM sources were negligible.

It is also possible that the small differences in SID of Lys and Trp among the 3 sources of SBM was caused by the small differences in trypsin inhibitors among the 3 sources. Elevated levels of trypsin inhibitors in SBM reduce SID of Lys and other AA (Goebel and Stein, 2011), but it is not known if the relative small differences in trypsin inhibitors among the 3 sources of SBM was sufficient to impact the SID of AA.

The negative effect of phytate on AA digestibility was demonstrated in the present experiment by the positive response to increasing levels of microbial phytase, which increased the digestibility of all AA. The increase in AID of CP and AA is in agreement with previous data, where microbial phytase increased AID of AA in pigs fed corn-SBM diets (Espinosa et al., 2022; Lagos et al., 2023). This response may be explained by the ability of phytate to reduce protein digestion by binding proteins and amino acids and by forming indigestible Ca–phytate–protein complexes binding the negatively charged phytate molecule and positively charged groups on Ca and proteins (Selle et al., 2012; Espinosa et al., 2022). As a consequence, hydrolysis of phytate by phytase reduces these anti-nutritional effects and increases digestibility of dietary protein and AA in pigs (Velayudhan et al., 2025).

Although the AID of AA was increased by microbial phytase in the present experiment and several other experiments, it has also been demonstrated that the AID is not always increased when phytase is used (She et al., 2018, Mesina et al., 2019). It is not clear why microbial phytase sometimes fail to increase SID of AA, but research to address this questions is warranted.

The increase in SID is important because SID values are corrected for basal endogenous losses and, therefore, better represent digestibility of AA in SBM than AID values (Stein et al.,

2007). The observation that SID of all AA increased with phytase indicates that phytase improved utilization of protein-bound AA in the diet and that the response was not only a consequence of reduced endogenous losses. This agrees with the concept that phytase may provide benefits beyond release of P and Ca (Stein et al., 2007; Dersjant-Li and Dusel, 2019; Lagos et al., 2023; Velayudhan et al., 2025).

The result that the response to phytase was consistent among the 3 sources of SBM has practical implications because it indicates that matrix values for digestible AA released by phytase may be used for SBM regardless of the sources used (Dersjant-Li et al., 2019). However, because only SBM that was produced in the U.S. was used in the present work, it is not known if the SID of AA in SBM from other origins also is increased by microbial phytase, but future research should be directed determining the impact of microbial phytase on the SID of AA in SBM produced outside the U.S.

Conclusions

Results indicate that source of SBM had only minor effects on ileal digestibility of CP and AA, whereas increasing phytase improved both AID and SID of all AA. Therefore, the main conclusion is that phytase improved the digestibility of protein and AA regardless of SBM source, which supports the hypothesis that the extra-phosphoric effects of phytase include improved AA utilization in SBM-based diets fed to growing pigs.

Disclosures

The authors have no conflicts of interest.

Tables

Table 6.1. Analyzed composition of feed ingredients, as-fed basis

Item	Soybean meal 01	Soybean meal 02	Soybean meal 03	Corn-starch	Calcium carbonate	Dicalcium phosphate
Dry matter, %	88.45	88.32	88.82	89.49	96.47	99.1
Gross energy, kcal/kg	4,136	4,119	4,135	4,156	-	-
Crude protein, %	47.37	45.08	46.50	-	-	-
Acid hydrolyzed ether extract, %	2.41	2.14	2.75	0.35	-	-
Ash, %	5.71	6.13	6.43	0.01	91.53	81.20
Phytic acid, %	1.51	1.50	1.42	-	-	-
Ca, %	0.25	0.40	0.57	-	39.13	24.54
P, %	0.69	0.69	0.67	-	0.02	19.05

Table 6.2. Analyzed amino acid composition, and trypsin inhibitor concentration in 3 sources of soybean meal, as-fed basis

Item	Soybean meal		
	01	02	03
Indispensable amino acids, %			
Arg	3.36	3.26	3.07
His	1.26	1.20	1.20
Ile	2.35	2.12	2.22
Leu	3.63	3.38	3.46
Lys	3.02	2.85	2.81
Met	0.66	0.59	0.61
Phe	2.45	2.29	2.38
Thr	1.79	1.70	1.71
Trp	0.64	0.61	0.61
Val	2.38	2.19	2.24
Dispensable amino acids, %			
Ala	2.03	1.93	1.93
Asp	5.34	4.92	5.00
Cys	0.71	0.61	0.62
Glu	8.97	8.69	8.27
Gly	2.01	1.84	1.91
Ser	1.94	2.04	1.84
Tyr	1.72	1.52	1.64
All amino acids, %	47.04	44.43	44.29
Lys:CP, %	6.38	6.32	6.04
Trypsin inhibitor, units/g	4,180	3,050	2,090

Table 6.3. Ingredient inclusion and expected nutrients of experimental diets, as-fed basis

Item%	Soybean meal			Nitrogen-free diet
	01	02	03	
Corn starch	61.30	61.47	61.56	95.76
Soybean meal 01	35.00	-	-	-
Soybean meal 02	-	35.00	-	-
Soybean meal 03	-	-	35.00	-
Dicalcium phosphate	1.25	1.25	1.26	2.00
Calcium carbonate[5]	0.65	0.48	0.38	0.44
Phytase premix[2]	0.50	0.50	0.50	0.50
Premix, vitamins + minerals[3]	0.50	0.50	0.50	0.50
Chromic oxide	0.40	0.40	0.40	0.40
Sodium chloride	0.40	0.40	0.40	0.40

[1]There were 3 sources of SBM. For each source of SBM there was one diet without phytase and 4 diets containing 500, 1,000, 2,000 or 4,000 units of phytase per kg.

[2]Five separate premixes were used to provide the levels of phytase in the final diets. For example, because the phytase source (Quantum Blue, AB Vista, Marlborough, UK) contains 5,000 FTU/g, the phytase premix for the diet containing 500 unit of phytase per kg was composed of 1% Quantum Blue and 99% corn-starch.

[3]The vitamin–mineral premix provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: vitamin A as retinyl acetate, 7,992 IU; vitamin D_3 as cholecalciferol, 1,598 IU; vitamin E as dl-α-tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfite, 1.50 mg; vitamin B_{12}, 0.03 mg; riboflavin, 6.00 mg; D-pantothenic acid as D-calcium pantothenate, 24.0 mg; niacin, 44.0 mg; biotin, 0.43 mg; folic acid, 2.0 mg; pyridoxine as

Table 6.3. (cont.)

pyridoxine hydrochloride, 8.0 mg; thiamine as thiamine mononitrate, 1.5 mg; Cu as basic copper carbonate, 20 mg; Fe as ferrous sulfate, 62.5 mg; I as ethylenediamine dihydroiodide, 0.50 mg; Mn as manganous oxide, 30 mg; Se as sodium selenite, 0.30 mg; and Zn as zinc carbonate, 125 mg per kilogram of complete diet.

Table 6.4. Analyzed nutrient composition of diets, as-fed basis

Item, %	Soybean meal 01					Soybean meal 02					Soybean meal 03					N-free diet
Phytase, units /kg	0	500	1,000	2,000	4,000	0	500	1,000	2,000	4,000	0	500	1,000	2,000	4,000	
Dry matter, %	88.58	88.52	88.82	89.95	89.09	89.20	89.01	88.90	89.02	88.99	89.82	90.28	90.28	89.96	90.02	88.36
GE[1], kcal/kg	3,767	3,791	3,798	3,805	3,819	3,800	3,749	3,809	3,805	3,801	3,798	3,783	3,824	3,832	3,792	3,540
CP[1], %	16.55	16.58	16.28	16.83	16.65	15.78	15.92	15.63	16.01	15.88	16.28	16.05	16.15	16.38	16.84	-
Ash, %	4.70	4.84	4.56	4.20	4.68	5.14	5.12	4.92	5.04	4.83	4.61	4.96	4.65	4.59	4.82	2.63
AEE[1], %	0.98	0.97	0.96	0.98	0.99	0.99	1.01	0.95	0.96	0.94	1.01	1.11	1.15	1.13	1.05	0.20
Ca, %	0.63	0.66	0.64	0.68	0.67	0.65	0.67	0.65	0.63	0.65	0.66	0.66	0.67	0.66	0.65	0.65
P, %	0.49	0.47	0.50	0.49	0.49	0.49	0.48	0.47	0.50	0.48	0.48	0.48	0.49	0.48	0.51	0.42
FTU[1]/kg	<70	650	1,200	2,600	5,700	<70	730	1,400	2,000	5,100	<70	530	920	2,400	5,700	<70
Indispensible AA[1], %																
Arg	1.01	1.18	0.87	1.13	0.98	1.05	1.16	1.09	1.02	1.16	0.95	0.82	1.1	1.17	1.14	0.01
His	0.39	0.45	0.34	0.43	0.37	0.40	0.45	0.41	0.39	0.44	0.37	0.31	0.43	0.44	0.43	-
Ile	0.72	0.83	0.63	0.81	0.69	0.73	0.82	0.75	0.74	0.83	0.65	0.55	0.75	0.77	0.76	0.03
Leu	1.15	1.34	1.01	1.30	1.12	1.20	1.34	1.24	1.19	1.33	1.06	0.89	1.22	1.27	1.24	0.04
Lys	0.95	1.1	0.83	1.06	0.91	0.96	1.07	0.99	0.94	1.06	0.89	0.94	1.01	1.04	1.02	0.03

Table 6.4 (cont.)

Met	0.19	0.22	0.17	0.21	0.19	0.19	0.21	0.19	0.19	0.21	0.18	0.15	0.20	0.20	0.20	-
Phe	0.76	0.88	0.66	0.86	0.74	0.79	0.89	0.83	0.79	0.88	0.71	0.59	0.81	0.84	0.83	0.02
Thr	0.57	0.66	0.51	0.64	0.55	0.59	0.66	0.62	0.57	0.64	0.53	0.44	0.60	0.63	0.62	0.01
Trp	0.15	0.14	0.22	0.22	0.21	0.19	0.19	0.16	0.19	0.21	0.20	0.15	0.19	0.18	0.21	<0.02
Val	0.75	0.87	0.65	0.84	0.72	0.77	0.86	0.79	0.78	0.87	0.70	0.58	0.80	0.83	0.81	0.02
Dispensable amino acids, %																
Ala	0.65	0.76	0.57	0.73	0.63	0.68	0.76	0.7	0.67	0.75	0.62	0.51	0.7	0.73	0.71	0.02
Asp	1.69	1.97	1.48	1.90	1.62	1.74	1.94	1.80	1.70	1.93	1.55	1.29	1.78	1.84	1.80	0.02
Cys	0.22	0.26	0.20	0.26	0.22	0.22	0.24	0.23	0.21	0.23	0.20	0.16	0.22	0.22	0.22	-
Glu	2.86	3.32	2.49	3.21	2.76	2.92	3.27	3.02	2.85	3.24	2.71	2.26	3.1	3.2	3.14	0.05
Gly	0.63	0.73	0.55	0.70	0.60	0.65	0.73	0.68	0.64	0.73	0.58	0.48	0.66	0.69	0.67	0.02
Ser	0.63	0.73	0.57	0.71	0.63	0.67	0.74	0.70	0.63	0.70	0.59	0.5	0.67	0.69	0.69	0.01
Tyr	0.43	0.51	0.37	0.49	0.41	0.47	0.51	0.49	0.46	0.52	0.38	0.35	0.43	0.49	0.48	0.02
All AA	14.9	17.2	13.18	16.73	14.5	15.4	17.1	15.85	15.07	16.94	13.94	11.74	15.84	16.44	16.16	0.77

[1] GE = gross energy; CP = crude protein; AEE = acid hydrolyzed ether extract; FTU = units of microbial phytase; AA = amino acids.

Table 6.5 Apparent ileal digestibility of crude protein (CP) and amino acids (AA) in diets containing 1 of 3 sources of soybean meal (SBM) graded levels of phytase in each source of SBM when fed to growing pigs[1,2]

	Soybean meal, source				*P*-value	Phytase, phytase unit/kg						*P*-value	
Item, %	01	02	03	SEM	SBM	0	500	1,000	2,000	4,000	SEM	Linear	Quadratic
Dry matter, %	78.4[a]	73.6[b]	77.4[a]	0.32	<0.001	73.9	75.9	77.6	76.0	78.9	0.418	<0.001	0.177
Crude protein	74.0	72.2	72.3	0.70	0.103	70.4	72.0	72.6	72.1	77.16	0.88	<0.001	0.276
Indispensable amino acids													
Arg	86.6	87.1	87.6	0.46	0.258	85.9	87.0	87.1	86.4	89.1	0.57	<0.001	0.237
His	80.7	80.6	81.4	0.59	0.160	79.6	80.6	80.8	80.0	83.7	0.73	<0.001	0.135
Ile	79.4	79.2	78.6	0.59	0.619	77.1	78.7	79.2	78.1	82.2	0.74	<0.001	0.291
Leu	79.1	78.9	78.8	0.56	0.921	77.0	78.4	79.1	78.0	82.1	0.71	<0.001	0.305
Lys	80.2	79.1	80.5	0.52	0.121	78.2	79.2	80.2	78.9	83.1	0.67	<0.001	0.206
Met	81.1	81.1	81.5	0.55	0.859	79.7	81.0	81.2	80.3	83.9	0.70	<0.001	0.212
Phe	78.9	78.9	78.5	0.58	0.862	76.8	78.2	78.9	77.9	82.1	0.74	<0.001	0.326
Thr	71.8	71.4	71.5	0.86	0.897	68.9	70.7	72.0	70.4	75.9	1.03	<0.001	0.337
Trp	81.8[a]	80.3[b]	80.0[b]	0.47	0.015	78.9	80.3	81.2	79.7	83.4	0.60	<0.001	0.290
Val	76.4	75.6	75.5	0.65	0.486	73.7	75.2	76.0	74.8	79.4	0.82	<0.001	0.253

Table 6.5 (cont.)

Dispensable amino acids													
Ala	72.3	71.1	71.3	0.85	0.446	69.5	70.6	71.5	70.2	76.0	1.04	<0.001	0.112
Asp	76.8	76.5	77.0	0.64	0.830	74.7	76.3	76.9	75.7	80.3	0.81	<0.001	0.237
Cys	68.0	65.9	66.5	1.16	0.195	64.1	65.9	67.1	65.4	71.6	1.36	<0.001	0.239
Glu	80.9	81.1	81.7	0.74	0.610	79.6	81.3	80.8	80.5	84.2	0.88	<0.001	0.251
Gly	64.6	62.2	63.2	1.22	0.358	59.6	62.6	62.7	61.8	69.77	1.56	<0.001	0.264
Ser	79.1	79.0	79.4	0.55	0.862	76.8	78.7	79.8	78.2	82.5	0.69	<0.001	0.511
Tyr	81.0	81.5	80.9	0.55	0.737	78.9	80.5	81.3	80.6	84.3	0.70	<0.001	0.657

[a-b] Means within a row lacking a common subscript letter are different ($P < 0.05$).

[1]Interactions between source of soybean meal and level of phytase were not significant. Therefore, only main effects are presented.

[2]The number of observations for each source of soybean meal was 40, whereas data for microbial phytase represent the mean of 24 observations.

Table 6.6 Standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in diets containing 1 of 3 sources of soybean meal (SBM) graded levels of phytase in each source of SBM when fed to growing pigs[1,2,3]

Item, %	Soybean meal, source				*P*-value	Phytase, phytase unit/kg						*P*-value	
	01	02	03	SEM	SBM	0	500	1,000	2,000	4,000	SEM	Linear	Quadratic
Dry matter, %	78.4[a]	73.6[b]	77.4[a]	0.32	<0.001	73.9	75.9	77.6	76.0	78.9	0.418	<0.001	0.177
Crude protein	89.9[ab]	88.2[b]	90.4[a]	0.69	0.050	87.0	88.6	89.3	88.8	93.8	0.88	<0.001	0.296
Indispensable amino acids													
Arg	93.3[y]	93.6[y]	94.7[x]	0.455	0.052	92.7	93.8	93.8	93.2	95.9	0.57	0.001	0.235
His	85.6	85.5	86.8	0.59	0.160	84.6	85.6	85.82	85.0	88.7	0.73	<0.001	0.134
Ile	84.2	83.9	84.2	0.59	0.901	82.1	83.7	84.2	83.1	87.2	0.74	<0.001	0.288
Leu	83.9	83.5	84.2	0.56	0.702	81.91	834	84.1	83.0	87.1	0.71	<0.001	0.304
Lys	84.7[ab]	83.5[b]	85.5[a]	0.52	0.025	82.9	83.9	84.9	83.6	87.7	0.67	<0.001	0.204
Met	85.5	85.6	86.5	0.55	0.377	84.4	85.7	85.8	84.9	88.6	0.70	<0.001	0.212
Phe	83.6	83.5	83.8	0.58	0.909	81.6	83.1	83.8	82.8	86.9	0.74	<0.001	0.325
Thr	81.1	80.2	81.6	0.86	0.340	78.3	80.1	81.4	79.9	85.3	1.03	<0.001	0.334
Trp	88.3[a]	86.8[b]	87.9[ab]	0.47	0.075	85.9	87.3	88.1	86.6	90.4	0.60	<0.001	0.286
Val	82.67	81.6	82.5	0.65	0.408	80.1	81.6	82.4	81.2	85.9	0.82	<0.001	0.252

Table 6.6 (cont.)

Dispensable amino acids													
Ala	81.8	80.1	81.6	0.85	0.223	79.1	80.2	81.1	79.8	85.6	1.04	<0.001	0.111
Asp	81.7	81.2	82.5	0.64	0.335	79.7	81.3	82.0	80.7	85.3	0.81	<0.001	0.236
Cys	76.6	75.5	77.3	1.16	0.303	73.1	75.7	76.9	75.1	81.4	1.34	<0.001	0.428
Glu	84.4	84.6	85.6	0.74	0.338	83.2	84.9	84.4	84.2	87.8	0.88	<0.001	0.251
Gly	87.9[ab]	84.5[b]	89.2[a]	1.22	0.020	83.5	86.5	86.5	85.7	93.7	1.56	<0.001	0.261
Ser	86.1	85.7	87.0	0.54	0.244	83.9	85.7	86.8	85.3	89.5	0.69	<0.001	0.518
Tyr	86.6	86.7	86.9	0.55	0.920	84.5	86.2	87.0	86.2	89.9	0.70	<0.001	0.653

[a-b] Means within a row lacking a common subscript letter are different ($P < 0.05$).

[x-y] Means within a row lacking a common subscript letter tend to be different ($P < 0.10$).

[1] Interactions between source of soybean meal and level of phytase were not significant. Therefore, only main effects are presented.

[2]Data for each source of soybean meal represent the mean of 40 observations, whereas data for microbial phytase represents the mean of 24 observations.

[3]Standardized ileal digestibility values were calculated by correcting apparent ileal digestibility values for basal endogenous amino acid losses of each individual amino acid and crude protein; crude protein, 29.34; Arg, 079; His; Ile 0.41; Leu, 0.66; Lys, 0.52; Met, 0.11; Phe, 0.43; Thr, 0.63; Trp, 0.15; Val, 0.55; Ala, 0.71; Asp 0.97; Cys, 0.25; Glu, 1.18; Gly, 1.64; Ser, 0.53; Tyr, 0.32.

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Chapter 7. Conclusions.

As pork production increases to meet future demand, continued work to better define the nutritional value of feed ingredients becomes imperative. That work should be based on digestible nutrient systems, particularly standardized total tract digestibility (**STTD**) for Ca, P and energy and standardized ileal digestibility (**SID**) for amino acids (**AA**) and fat, because these systems provide a more realistic estimate of nutrient availability to the pig than total nutrient concentrations.

Within that context, feed additives should be also considered formulation tools, but their contribution needs to be defined carefully. The common use of 25-hydroxycholecalciferol [**25(OH)D_3**] in pig diets reflects the interest in improving vitamin D availability because of its role in bone development and in Ca and P metabolism. Likewise, phytase is used to hydrolyze phytate and increase the availability of P and Ca. Results from this dissertation indicate that 25(OH)D_3 can improve Ca and P digestibility, but phytase alone increases the Ca and P in greater proportion but is not affected by 25(OH)D_3. However, responses such as increased osteocalcin and improved plasma vitamin D status indicate that both additives influence nutrient utilization, and additionally, physiological status.

The other 3 experiments had shown that the effect of phytase in pig diets extends beyond P release. Phytate does not only release bound P, but also interacts with Ca and AA, and may reduce the effective use of energy and other nutrients in the diet. Therefore, the positive responses observed with 2,000 units of phytase per kg in these experiments are better interpreted as a reduction of the anti-nutritional effects of phytate in diets using superdosing and containing SBM. Perhaps a matrix value of energy, Ca and AA, should be applied to the SBM when 2,000 units of phytase are supplemented,

In conclusion, this dissertation supports the use of feed additives as practical tools to improve the effective nutritional value of pig diets. At the same time, it also shows that greater precision in their application will require a better understanding of the mechanisms by which additives affect digestion, absorption, and metabolism, and more accurate estimates of the real release of nutrients and energy under different dietary conditions. That is necessary not only to assign matrix values with greater confidence, but also to continue improving the valuation of ingredients and the accuracy of practical diet formulation.