# Influence of soybean meal characteristics and feed enzyme technologies on nutrient utilization in pigs

by

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# Abstract

Soybean meal (**SBM**) is a common protein source in swine diets; however, SBM quality may be compromised by pauses or interruptions in thermal processing. In chapter 1, the objectives of the two studies were to determine the effects of startup SBM on standardized ileal digestibility (**SID**) of amino acids (**AA**) in grow-finishing pigs and to evaluate the effects of increasing startup SBM inclusion in nursery pig diets on growth performance, fecal dry matter (**DM**), and apparent total tract digestibility (**ATTD**) of crude protein (**CP**). Additionally, SBM contains an array of antinutritional factors; however, trypsin inhibitors specifically interfere with the digestion of protein. While thermal processing is utilized to minimize the effects of trypsin inhibitors, under processing may fail to inactivate trypsin inhibitors, while over processing may inactivate trypsin inhibitors, it may also reduce the availability of amino acids. In chapter 2, the objective was to evaluate the effects of exogenous protease on the SID of CP and AA in SBM sourced with differing trypsin inhibitor concentrations. Collectively, these studies demonstrated that the effects of pauses or interruptions in thermal processing may reduce the protein quality when fed to pigs but may also have age-specific effects in the early nursery phases. Additionally, the effect of protease supplementation may improve AA digestibility regardless of trypsin inhibitor concentration. Building on these findings, the effects of chapter 3 were to compare the effects of carbohydrase supplementation on the ATTD of nutrients, apparent metabolizable energy, and nitrogen retention in grow-finishing pigs fed corn and SBM-based diets. However, exogenous carbohydrase supplementation targeting NSP did not result in improved energy or nitrogen utilization in grow-finishing pigs, but did result in improvements of the ATTD of Ca.

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## Lay summary

Soybean meal is a common protein source in swine diets due to its favorable amino acid profile. However, soybeans naturally contain antinutritional factors that interfere with nutrient digestion. Thermal processing may deactivate some antinutritional factors; however, insufficient heat fails to inactivate these factors, while excessive heat reduces bioavailability of amino acids. Consequently, processing techniques may vary within each batch due to pauses or stops in production, which may cause variability in soybean meal quality. Two experiments were conducted to determine the effects of startup soybean meal on the standardized ileal digestibility of amino acids in grow-finishing pigs, and to evaluate the effects of increasing startup soybean meal on growth performance, fecal dry matter, and apparent total tract digestibility of crude protein in nursery pigs. In Exp 1. Pigs fed startup soybean meal had reduced amino acid digestibility compared with pigs fed standard soybean meal. In Exp. 2, increasing inclusions of startup soybean meal diets reduced growth and feed efficiency during phases 1 and 2; however, there were no effects observed in phase 3. These findings indicate that startup soybean meal, soybean meal produced after process interruptions, may reduce amino acid digestibility in soybean meal and impair performance during early development.

## Teaser text

Different soybean processing environments can lead to differences in soybean meal quality, reducing nutrient utilization and growth performance in swine.

## Abstract

Two experiments were conducted to evaluate the nutritional quality of startup soybean meal (**SBM**) across two production phases in swine. The objectives of these studies were to determine the effects of startup SBM on standardized ileal digestibility (**SID**) of amino acids (**AA**) in grow-finishing pigs and to evaluate the effects of increasing startup SBM inclusion in nursery pig diets on growth performance, fecal dry matter (**DM**), and apparent total tract digestibility (**ATTD**) of crude protein (**CP**). Both experiments used pigs from the same genetic line (DNA 241 × 600). In Exp 1, 12 ileal cannulated barrows (initial BW: 83.03 kg ± 3.54 kg) were allotted to a 4 x 4 Latin Square Design. Each experimental period consisted of 5d of dietary adaptation followed by 2d of ileal digesta collection (8h/d). There was no evidence that the AID of CP, His, and Gly differed between SBM sources. Pigs fed standard SBM had greater ($P < 0.05$) SID of Arg, His, Met, Phe, Asp, Glu, and Tyr and tended ($P < 0.10$) to have greater SID of Ile, Leu, and Lys compared with pigs fed the startup SBM. In Exp. 2, 300 pigs (initial BW 4.99 ± 0.004 kg) were weaned (19d of age) and randomly assigned to pens with five pigs per pen. From d 0 to 10, increasing the startup SBM tended to decrease (linear, $P < 0.10$) ADG and d 10 BW and caused poorer (linear, $P = 0.041$) F/G. From d 10 to 22, increasing startup SBM decreased (linear, $P < 0.05$) ADG and d 22 BW, with a tendency for worsening (linear, $P = 0.067$) in F/G. Additionally, increasing the startup SBM led to a tendency for decreased (linear, $P = 0.095$) ATTD of DM. No effects were observed for growth performance from d 22 to 38 and for the overall nursery period. In conclusion, startup soybean meal produced after process interruption may reduce AA digestibility and impair pig performance in early nursery phases.



**Key words:** growth performance, ileal digestibility, pigs, SBM

## List of abbreviations:

**AA,** amino acid;

**ADG,** average daily gain;

**ADFI,** average daily feed intake;

**AID,** apparent ileal digestibility;

**ATTD,** apparent total tract digestibility;

**CP,** crude protein;

**DM,** dry matter;

**SBM,** soybean meal;

**SID,** standardized ileal digestibility.

## Introduction

Soybean meal (**SBM**) is a widely used protein source in swine diets due to its favorable amino acid profile and relatively high protein digestibility. However, the nutritional value of SBM is highly dependent on the degree of thermal processing. Raw soybeans contain an array of antinutritional factors, including trypsin inhibitors, lectins, glycinin, β-conglycinin, phytic acid, saponins, oligosaccharides, and isoflavones, that compromise protein and amino acid digestibility (Liener, 1994; Wedekind et al., 2020). These antinutritional factors impair nutrient digestibility by inhibiting endogenous enzymes and reducing the efficiency of protein and nutrient breakdown in the gastrointestinal tract (Liener, 1994; Baker, 2000). Therefore, thermal processing is necessary to inactivate antinutritional factors in SBM; however, excessive thermal processing can reduce amino acid (AA) digestibility via the Maillard reaction (Parsons et al., 1992; Baker, 2000; González-Vega et al., 2011; Lagosadeol and Stein, 2017). Heat-induced reductions in AA digestibility limit the supply of AA for pigs, resulting in impaired growth

performance, poor feed efficiency, and reduced nitrogen retention (NRC, 2012; González-Vega et al., 2011). Lysine is particularly susceptible to these reactions because it contains a reactive ε-amino group that readily binds with reducing sugars, rendering it unavailable for absorption (Pahm et al., 2008).

Despite the well-documented effects of processing on SBM quality, variability in commercial processing conditions result in differences in SBM nutritional quality among sources (González-Vega et al., 2011; Son and Kim, 2023; Gaffield et al., 2024). Variability in SBM nutritional value is primarily driven by differences in processing conditions (Stein et al., 2008; Lagos and Stein, 2017; González-Vega et al., 2011). Differences in equipment design, operating parameters, control of temperature, type of heat applied (dry vs steam), duration of heat applied, and the feed ingredient being processed collectively influence variation in antinutritional factor inactivation and AA availability (Araba and Dale, 1990; González-Vega et al., 2011; Lagos and Stein, 2017). This variability has practical implications across swine production, but its consequences may differ depending on the physiological stage of the animal due to differences in digestive capacity, nutrient requirement, and sensitivity to dietary components (Pluske et al., 1997; NRC, 2012).

During the nursery phase, pigs have exceptionally high requirements for AA to support rapid growth and immune function characteristics of early post-weaning (NRC, 2012; González-Vega et al., 2011; Wu, 2014). Reductions in bioavailable AA from thermal processing may lead to reduced average daily gain (ADG) and poor feed efficiency (NRC, 2012; González-Vega et al., 2011; Wu, 2014; González-Vega et al., 2011). Additionally, antinutritional factors such as trypsin inhibitors may inhibit endogenous enzymes, trypsin and chymotrypsin, from breaking down protein, thereby limiting AA hydrolysis in nursery pigs (Liener, 1994; Baker, 2000). As a

result, greater proportions of undigested protein reach the hindgut, elevating ammonia production, increasing luminal pH, and contributing to post-weaning diarrhea (Pieper et al., 2016; Zhang et al., 2020; Pluske et al., 2002). Collectively, these factors are indications of reduced feed efficiency and impaired intestinal nutrient utilization in nursery pigs, which may compromise growth performance, ADG, and feed intake (Pluske et al., 2002; Pieper et al., 2016; Zhang et al., 2020). While the negative effects are more pronounced during the early nursery phase, residual impacts carry into the grow-finishing phase, potentially influencing overall growth performance (Hornick et a., 2000; González-Vega et al., 2011; Menegat et al., 2020).

In growing-finishing pigs, AA digestibility is a key determinant of protein deposition and overall growth efficiency, such that differences in SBM quality can influence production outcomes (Stein et al., 2007; González-Vega et al., 2011). Excessive thermal processing can reduce the apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of CP and AA due to heat-induced damage to protein structures and reduced AA availability (González-Vega et al., 2011; Stein et al., 2017). Lysine is the first-limiting amino acid in swine diets, meaning that its availability determines the efficiency of protein deposition and growth performance in pigs (Stein et al., 2007). Furthermore, the negative effects of trypsin inhibitors continue to reduce AA digestibility and negatively affect growth performance in grow-finishing pigs, underscoring the importance of SBM quality across all phases of production (Miller et al., 2025).

Therefore, two experiments were conducted to evaluate the effects of SBM processed at two separate time points from a single commercial soybean crushing plant. The startup soybean meal was collected from the initial truckload during the startup phase of production, while the standard soybean meal that was collected 4 hours after thermal processing was stabilized. The

objective of Exp. 1 was to determine SID of crude protein (CP) and AA of each SBM when fed to in grow-finishing pigs. The objective of Exp. 2 was to evaluate the effects of each SBM on growth performance and nutrient digestibility when fed to nursery pigs.

## Materials and methods

The Institutional Animal Care and Use Committee at Kansas State University approved the protocol of these experiments.

### Soybean meal

All soybean meal (**SBM**) was sourced from the same soybean processing facility following production shutdown for preventative maintenance. The first batch was obtained from the initial truckload from the startup phase of the plant. Approximately four hours later, a second batch was sourced when soybean meal processing had stabilized. Therefore, each SBM will be identified as the startup or standard SBM. Prior to each experiment, SBM samples were collected and sent to commercial lab for analyses as described below.

### Experiment 1: Amino Acid Digestibility

Dietary treatments were formulated using a semi-purified diet with either the startup or standard SBM as the sole source of protein and to meet or exceed all vitamin and mineral recommendations for pigs between 75 and 85 kg (**Table 1**; NRC, 2012). All diets were manufactured at the O.H. Kruse Feed Technology and Innovation Center in Manhattan, KS, USA. Batches of experimental diets were mixed using a 227 kg paddle mixer (Model S paddle mixer; H. C. Davis Sons Manufacturing Co., Inc.; Bonner Springs, KS, USA) (**Table 2**). After mixing, diets were bagged, and a representative sample was collected from each bag using a sample probe. Samples were then combined into a single composite sample. Composite samples were then sent to a commercial laboratory and analyzed as described below.

A total of 12 castrated male pigs (241 × 600, DNA Genetics, Columbus, NE, USA) were surgically equipped with a T-cannula in the distal ileum (Stein et al., 1998). Initially, dietary treatments consisted of a 2 × 2 factorial with soybean meal (startup vs standard) and a proprietary feed additive. Therefore, the animals were allotted to a triplicated 4 x 4 Latin square design (Kim and Stein, 2009) with four periods of 7 d and 4 dietary treatments. Therefore, each diet was fed to 3 pigs in each period for a total of 12 replicate pigs per dietary treatment for the 4 periods. However, the proprietary feed additive data was not utilized in this publication and was removed from the analysis. Therefore, treatments consisted of the startup and standard SBM and each treatment had 12 replicates.

Pigs were fed 3.0 times the maintenance requirement for metabolizable energy (**ME**, i.e., 197 kcal/kg body weight$^{0.60}$; NRC, 2012) and had ad libitum access to water throughout the experiment. The daily feed allowance was divided into two equal meals and fed at 0800 and 1600 h. On collection days, samples were collected for 8h by removing the lid of the cannula, attaching a plastic bag to the barrel of the cannula, and securing it with a plastic cable tie. The digesta flowing into the bag was collected every 30 minutes or every time the bag was full. Digesta samples were immediately stored at -20 °C to prevent bacterial degradation of AA.

At the conclusion of the experiment, digesta samples were thawed, subsampled, lyophilized, and ground in preparation for laboratory analysis. Samples of SBM were analyzed for Trypsin Inhibitor Units (**TIU**; method 22-40; AACC, 2006), KOH protein solubility (0.2% KOH; Araba and Dale, 1990), protein dispersibility index (**PDI**; method Ba 10-65; AOCS, 2009), ash (method 942.05; AOAC, 2005), Crude fiber (method 978.10; AOAC, 2005), crude fat (**CF**; method 920.29(A), AOCS, 2006), and Urease (method 22-90, AACC, 1999). Samples of SBM, diets, and ileal digesta were analyzed for dry matter (**DM**; method 930.15; AOAC, 1999)

and nitrogen using the combustion method in a LECO analyzer (method 990.03; AOAC, 2006). Crude protein (**CP**) was calculated as nitrogen × 6.25. Diet and ileal digesta samples were also analyzed for AA (method 982.30 E [a, b, c]; AOAC, 2006) (**Table 3**), and titanium was quantified following the method described by Myers et al. (2004).

Apparent ileal digestibility and SID of CP and AA were calculated for the two dietary treatments as described by Stein et al. (2007). The values for the basal endogenous losses of CP and AA used to calculate SID were obtained from Adeola et al., (2016).

## Experiment 2: Nursery Pig Growth Performance

The two different soybean meal (**SBM**) sources, previously described, were blended to create a titration of startup SBM in the diet. The nutritional composition of both SBM sources was considered the same, and the startup SBM replaced the normal soybean meal on an equal weight basis without changes to the formulation (**Table 4**). Therefore, dietary treatments included increasing levels of startup SBM, replacing 0, 25, 50, 75, and 100% of standard SBM in the complete diet.

A total of 300 pigs (DNA 241 × 600, initial BW 5.40 kg) were weaned at approximately 19 d of age and randomly assigned to pens with 5 pigs per pen. Pens were then randomly allotted to one of five dietary treatments in a generalized randomized complete block design with 12 replicate pens per treatment.

Pig weights and feed disappearance were measured on d 0, 10, 17, 22, 31, and 38 to determine average daily gain **(ADG)**, average daily feed intake **(ADFI)**, and gain:feed **(G:F)**. All diets were fed in meal form in three phases: phase 1 from weaning to d 10, phase 2 from d 10 to 22, and phase 3 from d 22 to 38. Feces were collected on d 10 and d 22 from three random pigs per pen to determine the percentage fecal DM. Additionally, titanium dioxide was included in

phase 2 diets as an indigestible marker to determine ATTD of DM and CP from samples collected on d 22.

Ground feed and fecal samples were dried in a 135°C drying oven for 2 h to determine percentage DM of the samples used for titanium analysis (AACC; method 22-40.01, 2006). Titanium dioxide concentration in dried feed and fecal samples was determined utilizing procedures outlined by Leone (1973). Feed and fecal samples were analyzed for DM (method 930.15; AOAC, 1999) and CP (AOAC 990.03, 2002) in the Kansas State University Swine Laboratory. The ATTD of DM and CP were determined using the index method described by Adeola (2001).

## Statistical Analyses

For Exp. 1, data were analyzed using the GLIMIX procedure of SAS (SAS Institute Inc., Cary, NC, USA) using pig as the experimental unit. Diet was the fixed effect and pig and period were random effects. Least square means were calculated for each independent variable. Studentized residuals were calculated for each observation. Observations with a studentized residual outside of ± 3 were considered outliers and removed.

For Exp 2., data were analyzed as a generalized randomized complete block design for a one-way ANOVA using the GLIMMIX procedure of SAS v. 9.4 (SAS Institute, Inc., Cary, NC). Pen was considered the experimental unit, room served as a random effect, and both treatment and block served as fixed effects. Linear and quadratic contrasts were used to test the main effects of increasing startup soybean meal. Similarly, contrasts were used to test for the main effects of treatment, day, and interaction between treatment and day on fecal DM. Result were considered significant with $P < 0.05$ and marginally significant with $P < 0.10$.

## Results

The analyzed nutrient composition for both soybean meal (**SBM**) sources was within expected ranges for analyzed nutrients, except for crude fat (NRC, 2012). The startup and standard SBM crude fat analysis were 3.75 and 3.10% , respectively, which were greater than anticipated. The startup SBM had a KOH solubility of 68.9% and a TIU of 6.34 $\pm$ 0.12 TIU/mg, while the standard SBM had a KOH solubility of 82.1% and a TIU of 7.12 $\pm$ 0.54 TIU/mg.

### Experiment 1

There was no evidence of differences between SBM for AID of CP, His, and Ser. However, the standard SBM had greater ($P < 0.05$ ) AID of Arg, Iso, Leu, Lys, Met, Phe, Thr, Trp, and Val, Ala, Asp, Cys, Glu, Gly, Tyr, mean indispensable AA, mean dispensable AA, and total AA compared with the startup SBM when fed to grow-finish pigs.

There was no evidence of differences ($P > 0.122$) between SBM for the SID of CP, Thr, Trp, Val, Ala, Cys, Gly, Ser, mean dispensable AA, and total AA when fed to grow-finish pigs. Pigs fed the standard SBM had greater ($P < 0.05$) SID of Arg, His, Met, and Phe, Asp, Glu, and Tyr and tended to have greater ($P < 0.10$) SID of Ile, Leu, Lys, and mean indispensable AA compared with startup SBM.

### Experiment 2

From d 0 to 10, increasing the startup SBM led to a marginal decrease in ADG and d 10 BW (linear, $P < 0.10$) and poorer (linear $P = 0.041$) G:F (Table 7). From d 10 to 22, increasing startup SBM decreased ADG and d 22 BW (linear, $P < 0.05$), with a marginal worsening (linear, $P = 0.067$) in G:F. However, no effects were observed for any growth performance criteria from d 22 to 38 and for the overall nursery period.

There was no evidence ($P = 0.892$) of an interaction between treatment and day for fecal DM. On d 10 and 22, fecal DM decreased (d 10, linear, $P = 0.053$; d 22, linear, $P = 0.045$) as startup SBM increased. There was no evidence for a main effect of day ($P = 0.204$) with pigs exhibiting fecal DM of approximately 20% for both sampling timepoints.

The ATTD of DM tended to decrease (linear, $P = 0.095$) as startup SBM increased in the diet. However, there was no evidence of a difference in ATTD of CP.

## Discussion

The conventional processing of soybeans into soybean meal (**SBM**) includes multiple stages such as dehulling, solvent extraction, and heat treatment to eliminate anti-nutritional factors (Wright, 1981). A soybean crush plant's startup phase is when the plant reinitiates its processing after a pause in operations. This is a critical phase in manufacturing since SBM quality is dependent on processing variables, meal processed during startup may differ in quality from meal produced under normal operations (Mustakas et al., 01981; Pope et al., 2007).

Previous research indicates that excessive heat treatment during soybean processing reduces nutrient availability, whereas insufficient heat treatment results in higher residual TIU, impairing proteolysis and reducing protein and AA digestibility (Knabe et al., 1987; González-Vega et al., 2011). Excessive heat leads to reactions between AA and reducing sugars, known as Maillard reactions, reducing their availability to the animal (Maillard, 1912). Although Maillard reactions can occur between reducing sugars and many AA, Lys is generally considered the AA most susceptible to heat damage. This susceptibility is due to the highly reactive ε-amino group located on the lysine side chain, which readily reacts with carbonyl groups of reducing sugars during thermal processing (Hannan and Lea, 1951, 1952). Arginine has also been identified as an

amino acid sensitive to heat processing and is typically considered the second most affected AA following Lys (Hulshof et al., 2016).

Previous research has used an autoclave to induce thermal stress on SBM to increase the formation of Maillard reaction products. When analyzing total AA content, the authors observed a decrease in Arg, Lys and Cys concentration, while CP and remaining AA were not influenced. However, the AID of CP and all AA were reduced in autoclaved SBM (González-Vega et al., 2011). In the experiment conducted herein, the startup SBM had decreased Lys and Arg content compared with the standard SBM, while CP and the remaining AA were similar, indicating the potential for the Maillard reaction.

In addition to the AA content, the SBM protein solubility in a KOH solution is analyzed to assess the protein solubility, which is an indirect indicator of processing severity and protein quality. Previous research has determined a target KOH protein solubility between 78 and 84%, where values below this range are associated with reduced nitrogen digestibility (Parsons et al., 1991). The SBM collected from the startup phase had a reduced KOH solubility (68.9%) compared with the standard SBM (82.1%). Therefore, the startup SBM was below the expected range of KOH protein solubility. However, the TIU and urease activity of both SBM were within the expected ranges.

Based on the SBM analyses, it is assumed that the startup SBM utilized during this experiment experience excessive heat treatment during the startup phase of soybean processing. The reduction in SBM protein quality during the startup phase was confirmed, as pigs fed the startup SBM had decreased AID and SID of AA. This decrease in AA digestibility was similar to the response observed in previous research when autoclaving SBM at 125ºC for 15 min (González-Vega et al., 2011).

Collectively, the SBM quality analyses and SID AA coefficients indicate that the startup SBM experienced a different processing environment resulting in a reduction of SBM quality, which corresponds with the early nursery growth performance response to increasing startup SBM inclusion. In contrast, previous research demonstrated that increasing the KOH solubility of SBM from 75 to 85% did not affect the growth performance of grower-finisher pigs (Smaniotto Losekann et al., 2025). Therefore, multiple factors, such as, the nutrient composition of the diet and stage of production, can influence the growth performance response observed due to variations in SBM quality.

Newly weaned pigs have immature gastrointestinal systems characterized by reduced enzyme secretion and absorptive capacity, which limits nutrient utilization (Campbell et al., 2013). They also have a high protein deposition in relation to their low feed intake. Therefore, it is important to provide adequate concentrations of AA from highly digestible protein sources (Goodband et al., 2023). Although this is needed to meet the needs for protein deposition, excess crude protein can create additional issues in nursery pigs. An increase in undigested proteins in the large intestines lead to increased microbial fermentation, which pose risk for post weaning diarrhea (Heo et al., 2013). Therefore, feeding a lower-quality protein source, such as the startup SBM, can potentially lead to issues in addition to poor growth performance. Although there were no observations of post weaning diarrhea, increasing the inclusion of startup SBM in the diet reduced the fecal DM. This can be explained by the reduced AA digestibility in the startup SBM likely resulted in greater flow of undigested protein to the hindgut. The phase-specific performance data indicates that nursery pigs are the most sensitive to SBM protein quality during the early post-weaning period, when digestive capacity is more limited and dietary protein quality is most critical for supporting rapid growth.

In summary, the quality of startup SBM can affect the AA digestibility of grow-finish pigs and performance of nursery pigs. More specifically, it reduced performance during the first three weeks after weaning. This suggests that nursery pigs may be particularly sensitive to SBM quality within the first three weeks post weaning.

## Conflict of interest

The authors declare no conflicts of interest.

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# List of tables

Table 1.1. Analyzed composition of soybean meal sources (as – fed basis)

| Item, % | Startup Soybean meal[1] | Standard Soybean meal[1] |
|---|---|---|
| Dry Matter, % | 88.5 | 89.7 |
| Crude Fat, % | 3.75 | 3.10 |
| Crude Fiber, % | 3.71 | 3.01 |
| Ash, % | 6.10 | 5.86 |
| Crude Protein, % | 46.3 | 45.5 |
| Protein Dispersibility Index, % | 18.76 | 22.02 |
| Protein Solubility (KOH), % | 68.9 | 82.1 |
| Trypsin Inhibitor (TIU/mg) | 6.34 | 7.12 |
| Urease | 0.09 | 0.13 |
| Indispensable AA, % | | |
| Arginine | 3.24 | 3.28 |
| Histidine | 1.17 | 1.20 |
| Isoleucine | 2.14 | 2.17 |
| Leucine | 3.42 | 3.48 |
| Lysine | 2.87 | 2.94 |
| Methionine | 0.58 | 0.60 |
| Phenylalanine | 2.31 | 2.33 |
| Threonine | 1.71 | 1.77 |
| Tryptophan | 0.55 | 0.55 |
| Valine | 2.24 | 2.28 |
| Dispensable AA, % | | |
| Alanine | 1.92 | 1.98 |
| Aspartic acid | 4.97 | 5.06 |
| Cysteine | 0.65 | 0.66 |
| Glutamic acid | 8.21 | 8.46 |
| Glycine | 1.85 | 1.90 |
| Serine | 1.89 | 1.97 |
| Tyrosine | 1.64 | 1.67 |

[1]All soybean meal was sourced from the same soybean crush plant. The first batch was obtained from the initial truckload from the startup phase of the plant. Approximately four hours later, a second batch was sourced when the soybean meal processing had stabilized.

Table 1.2. Ingredient composition of the diets for Exp. 1 (as is basis)

| Ingredients, % | Startup Soybean meal[1] | Soybean meal[1] |
|---|---|---|
| Soybean meal | 33.00 | 33.00 |
| Corn starch | 41.94 | 41.94 |

| | | |
|---|---|---|
| Soybean oil | 2.00 | 2.00 |
| Monocalcium phosphate | 1.20 | 1.20 |
| Calcium carbonate | 0.60 | 0.60 |
| Sucrose | 20.00 | 20.00 |
| Sodium chloride | 0.50 | 0.50 |
| Vitamin premix[2] | 0.125 | 0.125 |
| Trace Mineral premix[3] | 0.125 | 0.125 |
| Phytase[4] | 0.01 | 0.01 |
| Titanium Dioxide | 0.50 | 0.50 |
| Total | 100.00 | 100.00 |

[1]All soybean meal was sourced from the same soybean crush plant. The first batch was obtained from the initial truckload from the startup phase of the plant. Approximately four hours later, a second batch was sourced when the soybean meal processing had stabilized.
[2]Provided per kg of diet: 110 mg of Zn from zinc sulfate; 110 mg of Fe from iron sulfate; 33 mg of Mn from manganese oxide; 17 mg of Cu from copper sulfate; 0.30 mg of Se from sodium selenite; 0.30 mg of I from calcium iodate,
[3]Provided per kg of diet: 4,134 IU vitamin A; 1,653 IU vitamin $D_3$; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin $B_{12}$; 50 mg of niacin; 28 mg pantothenic acid; 8 mg riboflavin.
[4]Quantum Blue 10g (AB Vista, Marlborough, Wiltshire, UK) contained 10,000 FTU/g. At 0.01% inclusion, complete diets were expected to contain 500 FTU/kg with an estimated release of 0.10% STTD P.

Table 1.3. Analyzed nutritional composition of experimental diets used in Exp. 1 (as-fed basis)[1]

| Item | Startup soybean meal[1] | Soybean meal[1] |
|---|---|---|
| Dry matter, % | 93.04 | 91.75 |
| Crude protein,[4] % | 15.85 | 17.94 |
| Indispensable AA, % | | |
| Arginine | 0.98 | 1.20 |
| Histidine | 0.37 | 0.44 |
| Isoleucine | 0.67 | 0.80 |
| Leucine | 1.06 | 1.29 |
| Lysine | 0.90 | 1.09 |
| Methionine | 0.19 | 0.23 |
| Phenylalanine | 0.71 | 0.86 |
| Threonine | 0.54 | 0.65 |
| Tryptophan | 0.18 | 0.21 |
| Valine | 0.72 | 0.85 |
| Dispensable AA, % | | |
| Alanine | 0.62 | 0.74 |
| Aspartic acid | 1.58 | 1.90 |
| Cysteine | 0.21 | 0.25 |
| Glutamic acid | 2.59 | 3.14 |
| Glycine | 0.60 | 0.71 |
| Serine | 0.61 | 0.73 |
| Tyrosine | 0.44 | 0.55 |

[1]All soybean meal was sourced from the same soybean crush plant. The first batch was obtained from the initial truckload from the startup phase of the plant. Approximately four hours later, a second batch was sourced when the soybean meal processing had stabilized.

Table 1.4. Diet composition for Exp. 2 (as-fed basis)[1]

| Ingredient, % | Phase 1 | Phase 2 | Phase 3 |
|---|---|---|---|
| Corn | 48.18 | 54.06 | 65.55 |
| Soybean meal[2] | 19.66 | 28.46 | 30.54 |
| Spray-dried whey | 10.00 | - | - |
| Whey permeate, 80% lactose | 10.00 | 10.00 | - |
| Enzymatically treated soybean meal[3] | 5.75 | 2.50 | - |
| Spray-dried bovine plasma | 2.50 | - | - |
| Monocalcium P, 21% P | 1.15 | 1.40 | 0.95 |
| Calcium Carbonate | 0.58 | 0.65 | 0.85 |
| Zinc Oxide | 0.40 | 0.25 | - |
| Salt | 0.40 | 0.50 | 0.60 |
| Vitamin premix | 0.25 | 0.25 | 0.25 |
| Trace mineral premix | 0.15 | 0.15 | 0.15 |
| Phytase[4] | 0.08 | 0.08 | 0.08 |
| L-Lys HCL | 0.40 | 0.52 | 0.48 |
| DL-Met | 0.21 | 0.24 | 0.20 |
| L-Thr | 0.18 | 0.25 | 0.22 |
| L-Trp | 0.02 | 0.04 | 0.04 |
| L-Val | 0.10 | 0.15 | 0.12 |
| L-Ile | - | 0.02 | - |
| Titanium dioxide | - | 0.50 | - |
| Total | 100 | 100 | 100 |
| Calculated Analysis | | | |
| SID amino acids, % | | | |
| Lys | 1.35 | 1.35 | 1.35 |
| Ile:Lys | 56 | 56 | 56 |
| Leu:Lys | 114 | 109 | 115 |
| Met: Lys | 35 | 38 | 36 |
| Met and Cys: Lys | 58 | 58 | 58 |
| Thr:Lys | 64 | 64 | 63 |
| Trp:Lys | 19.2 | 19.3 | 19.3 |
| Val:Lys | 70 | 70 | 70 |
| Total Lys, % | 1.50 | 1.49 | 1.50 |
| NE, kcal/kg | 2,487 | 2,432 | 2,418 |
| SID Lys:NE, g/Mcal | 5.43 | 5.55 | 5.58 |
| CP, % | 20.7 | 20.7 | 21.3 |
| Ca,% | 0.70 | 0.72 | 0.71 |
| P, % | 0.69 | 0.70 | 0.61 |
| STTP P, % | 0.60 | 0.58 | 0.48 |

[1]Phase 1 diets were fed from 5.4 kg to 6.5 kg, phase 2 diets were fed from 6.5 kg to 11.2 kg, and phase 3 diets were fed from 11.2 kg to 20.3 kg.
[2]All soybean meal was sourced from the same soybean crush plant. The first batch was obtained from the initial truckload from the startup phase of the plant. Approximately four hours later, a second batch was sourced when the soybean meal processing had stabilized. The nutritional composition of both soybean meal sources was considered the same, and thus the startup soybean meal replaced the normal soybean meal on an equal weight basis without other changes to the formulation.
[3]HP 300, Hamlet Protein, Findlay, OH.
[4]Ronozyme HiPhos 2700 GT, DSM Nutritional Products, Parsippany, NJ was included at 2,000 FTU/kg to provide an estimated release of 0.12% STTD P for all diets.

Table 1.5. Effects of soybean meal source on apparent ileal digestibility (AID) of crude protein and amino acids (AA).

| Soybean meal: | Startup Soybean meal[1] | Soybean meal[1] | SEM | *P* < |
|---|---|---|---|---|
| AID, % | | | | |
| Crude protein | 74.3 | 76.3 | 1.16 | 0.15 |
| Indispensable AA | | | | |
| Arginine | 82.7 | 86.2 | 0.90 | 0.024 |
| Histidine | 77.9 | 81.2 | 1.11 | 0.150 |
| Isoleucine | 73.7 | 76.9 | 1.18 | 0.007 |
| Leucine | 73.7 | 76.9 | 1.19 | 0.015 |
| Lysine | 77.3 | 80.4 | 1.11 | 0.022 |
| Methionine | 76.6 | 79.9 | 1.04 | 0.020 |
| Phenylalanine | 73.7 | 77.3 | 1.20 | 0.027 |
| Threonine | 67.6 | 71.2 | 1.42 | 0.006 |
| Tryptophan | 81.5 | 82.0 | 1.14 | 0.017 |
| Valine | 71.5 | 74.7 | 1.29 | 0.033 |
| Mean | 75.4 | 78.7 | 1.13 | 0.021 |
| Dispensable AA | | | | |
| Alanine | 67.3 | 70.8 | 1.43 | 0.029 |
| Aspartic acid | 73.2 | 77.0 | 1.28 | 0.011 |
| Cysteine | 63.4 | 67.5 | 1.85 | 0.035 |
| Glutamic acid | 76.8 | 80.4 | 1.30 | 0.017 |
| Glycine | 63.7 | 67.5 | 1.81 | 0.046 |
| Serine | 75.5 | 78.2 | 1.06 | 0.128 |
| Tyrosine | 71.7 | 76.6 | 1.25 | 0.024 |
| Mean | 72.2 | 76.1 | 1.38 | 0.021 |
| Total AA | 73.7 | 77.3 | 1.25 | 0.021 |

[1]All soybean meal was sourced from the same soybean crush plant. The first batch was obtained from the initial truckload from the startup phase of the plant. Approximately four hours later, a second batch was sourced when the soybean meal processing had stabilized.

Table 1.6. Effects of soybean meal source on standardized ileal digestibility (SID) of crude protein and amino acids (AA).

| Soybean meal: | Startup Soybean meal[1] | Soybean meal[1] | SEM | *P* < |
|---|---|---|---|---|
| SID, % | | | | |
| Crude protein | 84.5 | 85.1 | 1.16 | 0.606 |
| Indispensable AA | | | | |
| Arginine | 88.4 | 90.7 | 0.90 | 0.042 |
| Histidine | 82.2 | 84.8 | 1.11 | 0.045 |
| Isoleucine | 77.8 | 80.4 | 1.18 | 0.061 |
| Leucine | 77.7 | 80.5 | 1.19 | 0.057 |
| Lysine | 81.4 | 83.8 | 1.11 | 0.074 |
| Methionine | 82.2 | 84.4 | 1.04 | 0.033 |
| Phenylalanine | 77.9 | 80.8 | 1.20 | 0.048 |
| Threonine | 76.7 | 78.6 | 1.42 | 0.204 |
| Tryptophan | 88.3 | 87.8 | 1.14 | 0.654 |
| Valine | 77.5 | 79.6 | 1.29 | 0.152 |
| Mean | 80.5 | 82.9 | 1.13 | 0.075 |
| Dispensable AA | | | | |
| Alanine | 75.9 | 77.9 | 1.43 | 0.174 |
| Aspartic acid | 77.7 | 80.6 | 1.28 | 0.031 |
| Cysteine | 71.0 | 73.8 | 1.85 | 0.122 |
| Glutamic acid | 80.2 | 83.1 | 1.30 | 0.039 |
| Glycine | 86.3 | 86.4 | 1.81 | 0.958 |
| Serine | 85.4 | 86.4 | 1.06 | 0.352 |
| Tyrosine | 79.2 | 82.5 | 1.25 | 0.040 |
| Mean | 84.7 | 86.3 | 1.38 | 0.282 |
| Total AA | 82.8 | 84.7 | 1.25 | 0.164 |

[1]All soybean meal was sourced from the same soybean crush plant. The first batch was obtained from the initial truckload from the startup phase of the plant. Approximately four hours later, a second batch was sourced when the soybean meal processing had stabilized.

Table 1.7. Effects of increasing startup soybean meal on nursery pig performance, fecal dry matter (DM), and *apparent total tract digestibility* (ATTD) of crude protein (CP) and DM[1]

| | Startup soybean meal,%[2] | | | | | | *P* = | |
|---|---|---|---|---|---|---|---|---|
| | **0** | **25** | **50** | **75** | **100** | **SEM** | **Linear** | **Quadratic** |
| BW, lb | | | | | | | | |
| d 0 | 11.8 | 11.8 | 11.8 | 11.8 | 11.8 | 0.01 | 0.658 | 0.723 |
| d 10 | 14.7 | 14.4 | 14.3 | 14.3 | 14.2 | 0.22 | 0.096 | 0.583 |
| d 22 | 25.7 | 24.7 | 24.7 | 24.6 | 24.0 | 0.40 | 0.012 | 0.712 |
| d 38 | 45.9 | 44.6 | 45.2 | 43.9 | 43.7 | 0.94 | 0.091 | 0.949 |
| d 0 to 10 (Phase 1) | | | | | | | | |
| ADG, lb | 0.29 | 0.27 | 0.25 | 0.26 | 0.24 | 0.021 | 0.097 | 0.593 |
| ADFI, lb | 0.32 | 0.32 | 0.30 | 0.31 | 0.30 | 0.021 | 0.493 | 0.855 |
| F/G | 1.09 | 1.20 | 1.26 | 1.27 | 1.28 | 0.067 | 0.041 | 0.346 |
| d 10 to 22 (Phase 2) | | | | | | | | |
| ADG, lb | 1.00 | 0.93 | 0.94 | 0.94 | 0.89 | 0.028 | 0.021 | 0.878 |
| ADFI, lb | 1.36 | 1.35 | 1.32 | 1.35 | 1.29 | 0.047 | 0.232 | 0.691 |
| F/G | 1.37 | 1.46 | 1.41 | 1.45 | 1.45 | 0.038 | 0.067 | 0.354 |
| d 22 to 38 (Phase 3) | | | | | | | | |
| ADG, lb | 1.19 | 1.15 | 1.15 | 1.15 | 1.19 | 0.031 | 0.996 | 0.155 |
| ADFI, lb | 1.72 | 1.70 | 1.67 | 1.70 | 1.72 | 0.043 | 0.897 | 0.397 |
| F/G | 1.44 | 1.47 | 1.46 | 1.49 | 1.44 | 0.025 | 0.818 | 0.240 |
| d 0 to 38 (Overall) | | | | | | | | |
| ADG, lb | 0.90 | 0.85 | 0.85 | 0.85 | 0.85 | 0.021 | 0.175 | 0.238 |
| ADFI, lb | 1.25 | 1.23 | 1.20 | 1.23 | 1.22 | 0.032 | 0.605 | 0.618 |
| F/G | 1.39 | 1.44 | 1.42 | 1.45 | 1.43 | 0.022 | 0.105 | 0.109 |
| Fecal DM, % | | | | | | | | |
| d 10 | 22.72 | 23.37 | 21.95 | 19.85 | 21.33 | 1.070 | 0.053 | 0.793 |
| d 22 | 20.78 | 20.97 | 19.72 | 19.07 | 19.19 | 0.853 | 0.045 | 0.869 |
| ATTD, % | | | | | | | | |
| DM | 83.81 | 82.96 | 83.58 | 81.44 | 82.87 | 0.957 | 0.095 | 0.455 |
| CP | 74.07 | 73.93 | 74.78 | 74.12 | 74.47 | 1.777 | 0.798 | 0.906 |

[1]Phase 1 diets were fed from 5.4 kg to 6.5 kg, phase 2 diets were fed from 6.5 kg to 11.2 kg, and phase 3 diets were fed from 11.2 kg to 20.3 kg.

[2]All soybean meal was sourced from the same soybean crush plant. The first batch was obtained from the initial truckload from the startup phase of the plant. Approximately four hours later, a second batch was sourced when the soybean meal processing had stabilized. The nutritional composition of both soybean meal sources was considered the same, and thus the startup soybean meal replaced the normal soybean meal on an equal weight basis without other changes to the formulation.

# Chapter 2 - Effect of exogenous protease on standardized ileal digestibility of amino acids in soybean meal with varying concentrations of trypsin inhibitors fed to grow-finishing pigs

## Lay summary

Although soybean meal (SBM) is a favorable protein source in swine diets, soybeans naturally contain antinutritional factors, most notably trypsin inhibitors. Trypsin inhibitors (TI) bind to digestive enzymes, preventing protein digestion and thereby negatively affecting nutrient absorption and utilization. The objective of this experiment was to determine if adding exogenous protease to diets could improve nutrient digestibility in pigs fed soybean meal with varying levels of TI. An interaction between SBM source and added protease was observed for the SID of Cys, Met, and Phe. Added protease improved the standardized ileal digestibility (SID) of Cys and tended to improve Met and Phe in pigs fed the high TI SBM, whereas pigs fed the low TI SBM had minimal improvements. Additionally, protease supplementation improved and tended to improve the SID of several amino acids and, interestingly, it also improved the AID of DM and tended to improve the AID of GE regardless of SBM source. Collectively, these findings may indicate that protease supplementation improved amino acid digestibility in pigs.

## Teaser text

Adding an exogenous protease improved the digestion of amino acids in two soybean meal sources; however, the level of trypsin inhibitors in the soybean meal had minimal effects on standardized ileal digestibility of protein or amino acids.

## Abstract

The thermal processing of soybean meal (SBM) can improve amino acid (AA) digestion by reducing antinutritional factors or, if over processed, can reduce its protein quality by decreasing solubility. Supplementation of exogenous protease may serve as a nutritional strategy to mitigate digestibility losses associated with trypsin inhibitor (TI) activity. The objective of this experiment was to evaluate the effects of exogenous protease on standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in SBM with differing TI concentrations when fed to pigs. Twelve ileal cannulated barrows (DNA 241 × 600; BW: 83.03 kg ± 3.54 kg) were allotted to a triplicated 4 × 4 Latin square design with 4 treatments and 4 experimental periods, resulting in 12 replicate pigs per treatment. Each experimental period consisted of 5d of dietary adaptation followed by 2d of ileal digesta collection (8h/d). Treatments were arranged in a 2 × 2 factorial with two SBM sources differing in trypsin inhibitor concentration (low, 1.72 TIU/mg or high, 5.25 TIU/mg) and two levels of protease supplementation (with or without CIBENZA DP100; Novus International, St. Charles, MO, USA). There was no evidence of an interaction between SBM source and protease supplementation for the SID of CP and most AA, except for the SID of Cys ($P = 0.025$) and a marginally significant interaction ($P \leq 0.094$) for the SID of Met and Phe. Added protease improved the SID of Cys and tended to improve the SID of Met and Phe in pigs fed the high TI SBM, while pigs fed the low TI SBM had minimal improvements. For the main effect of SBM source, the low TI SBM had greater ($P < 0.034$) SID of Phe and Trp, and a tendency for greater SID of Leu ($P = 0.082$), compared with pigs fed the high TI SBM. Regardless of SBM source, protease supplementation improved ($P < 0.05$) SID of Arg, Ile, Leu, Val, Ala, Cys, Glu, Ser, indispensable AA, and tended to improve ($P < 0.10$) SID of Met, Phe, Thr, Asp, and total AA. Additionally, the inclusion of protease improved the

apparent ileal digestibility (AID) of dry matter (DM) and tended to increase ($P < 0.10$) AID of gross energy (GE). In conclusion, protease supplementation improved AA digestibility regardless of SBM source.

**Key words:** trypsin inhibitors, amino acids, digestibility, exogenous protease, pigs

## List of abbreviations:

**AA,** amino acid;

**AID,** apparent ileal digestibility;

**ATTD,** apparent total tract digestibility;

**CP,** crude protein;

**DE,** digestible energy;

**DM,** dry matter;

**GE**, gross energy;

**ME,** metabolizable energy;

**PDI,** Protein dispersibility index;

**SID,** standardized ieal digestibility;

**TI,** trypsin inhibitor;

**TIU**, trypsin inhibitor units;

**SBM,** soybean meal.

## Introduction

Soybean meal (**SBM**) is the most widely used protein source in swine diets because of its high protein content and favorable amino acid (**AA**) profile. Although SBM is a favorable protein source, soybeans contain an array of naturally occurring antinutritional factors, including protease inhibitors, lectins, glycinin, β-conglycinin, phytic acid, saponins, oligosaccharides, and

isoflavones that can impede nutrient absorption and utilization in monogastric animals (Liener, 1994; Friedman and Brandon, 2001; Mishra et al., 2024). The concentration of these antinutritional factors in SBM can vary depending on processing conditions, as factors such as temperature, moisture, and processing time influence the effectiveness of heat treatment and the subsequent reduction of these compounds. Trypsin inhibitors (**TI**) and lectins are the most nutritionally significant because they exert the most direct negative effect on protein utilization and growth performance, while phytic acid, oligosaccharides, saponins, and isoflavones are generally considered of lesser consequence in properly processed SBM fed to swine (Liener, 1994).

Trypsin inhibitors have a negative effect on animal performance by inhibiting protease enzymes in the digestive tract (Liener, 1976; Goebel and Stein, 2011). Specifically, they reduce trypsin and chymotrypsin activity, which leads to impairment of protein digestion in swine (Norton, 1991). Kunitz TI and Bowman-Birk Inhibitor are the two TI that are found in soybeans (Kunitz, 1946; Bowman, 1944; Birk, 1961). Recent research has concluded that as the intake of TI increased, pig growth performance, feed efficiency, and nitrogen digestibility and retention were negatively impacted. Suggesting that diets containing more than 3.0 TIU/mg may start to reduce pig performance (Miller et al., 2025).

The conventional processing of soybeans into SBM includes multiple stages such as dehulling, solvent extraction, and heat treatment to remove moisture and eliminate anti-nutritional factors (Liener, 1994;Deng and Kim, 2024). During thermal processing, adequate temperature, moisture, and residence time are required to ensure effective inactivation of TI while minimizing nutrient damage (Avilés-Gaxiola et al., 2018; Oliveira et al., 2020). Insufficient processing parameters fail to adequately inactivate TI, whereas excessive processing

parameters can negatively impact other essential nutrients (Avilés-Gaxiola et al., 2018). For instance, over-processing can lead to the Maillard reaction, which occurs when reducing sugars covalently bind to the amino groups of proteins, thereby reducing lysine bioavailability and impairing overall AA digestibility (González-Vega et al., 2011). Furthermore, excessive heat treatment induces protein aggregation and degrades sulfur-containing AA, thereby diminishing the nutritional value of the over-processed SBM (González-Vega et al., 2011; Ju et al., 2023). An industry-wide survey determined that trypsin inhibitor units (**TIU**) varied from 1.45 to 9.26 TIU/mg across SBM samples from 14 different plants. This demonstrates differences in processing procedures and incoming soybean quality that results in various concentrations of TIU (Gaffield et al. 2024).

Given the variability in residual TIU across commercially processed SBM, supplementation of exogenous protease has emerged as a practical nutritional strategy to mitigate the digestibility losses associated with TI (Galli et al., 2024). Exogenous proteases are structurally distinct from endogenous trypsin and chymotrypsin and may not be susceptible to inhibition by soybean TI, enabling them to maintain full proteolytic activity under conditions where endogenous enzyme function is compromised (Aderibigbe et al., 2021). However, it is important to note that the protease used in this experiment also contained keratinase activity, which may disrupt the disulfide-bonds in trypsin inhibitors, which is different from using a conventional protease. Previous research has demonstrated that adding a serine protease with keratinase activity to diets with elevated TI concentrations improved protein digestion in broilers (Wang et al., 2008). Therefore, the objective of this study was to evaluate the effects of exogenous protease on standardized ileal digestibility (**SID**) of crude protein (**CP**) and AA in SBM with differing TI concentrations when fed to pigs.

## Materials and methods

The Institutional Animal Care and Use Committee at Kansas State University approved the protocol of this experiment.

### Soybean meal

All soybean meal was sourced from two separate commercial crushing facilities approximately around the same time of the crop year.

### Diet and diet preparation

Experimental treatments were arranged in a 2 × 2 factorial with two SBM sources differing in TI concentration (low; 1.72 TIU mg/g or high; 5.25 TIU mg/g; **Table 1**) and two levels of protease supplementation (with or without CIBENZA DP100; Novus International, St. Charles, MO, USA). The serine protease with keratinase activity (Cibenza DP100, NOVUS International Inc.) was included at 300 U protease activity/g of feed. A semi-purified diet based on SBM was formulated, so that soybean meal was the sole source of protein (**Table 2**) and met or exceed all vitamin and mineral requirements for pigs between 40 and 65 kg (NRC, 2012). All diets were manufactured at the O.H. Kruse Feed Technology and Innovation Center in Manhattan, KS, USA. Batches of the experimental diets were mixed using a 227 kg paddle mixer (Model S paddle mixer; H. C. Davis Sons Manufacturing Co., Inc.; Bonner Springs, KS). After mixing, diets were bagged, and a representative sample was collected from each bag using a sample probe. Samples were then combined into a single composite sample.

### Amino acid digestibility

A total of 12 barrows (average initial body weight: 37.2 ± 1.9 kg; 241 × 600, DNA Genetics, Columbus, NE, USA) were surgically equipped with a T-cannula in the distal ileum (Stein et al., 1998). The animals were allotted to a triplicated 4 × 4 Latin square design (Kim and

Stein, 2009) with four periods of 10 d and 4 dietary treatments. Therefore, each diet was fed to 3 pigs in each period for a total of 12 replicate pigs per dietary treatment for the 4 periods. Pigs were fed 3.0 times the maintenance requirement for metabolizable energy (**ME**, i.e., 197 kcal/kg body weight$^{0.60}$; NRC, 2012) and had free access to water throughout the experiment. The daily feeding allowance was divided into two equal meals and fed at 0730 and 1630 h.

The first six days of each period were considered the adaptation period to the diet (Adeola, 2001), with the fecal sample collection taking place on days 7 and 8 and ileal sample collection taking place on days 9 and 10. On each fecal collection day, samples were collected at 0730 and 1630 h by grabbing freshly voided feces directly from the rectum and immediately stored at -20 °C. Samples collected across both collection days were combined into a single composite sample per pig. On each digesta collection day, samples were collected for 8 h by removing the lid of the cannula, attaching a plastic bag to the barrel of the cannula, and securing it with a plastic cable tie. The digesta flowing into the bag was collected every 30 minutes or every time the bag was full. Digesta samples were immediately stored at -20°C to prevent bacterial degradation of AA.

At the conclusion of the experiment, digesta samples were thawed, subsampled, lyophilized, and ground in preparation for laboratory analysis. Samples of SBM were analyzed for TIU (**TIU**; method 22-40; AACC, 2006), KOH protein solubility (0.2% KOH; Araba and Dale, 1990), and protein dispersibility index (**PDI**; Method Ba 10-65; AOCS, 2009). Samples of SBM, diets, ileal digesta, and fecals were analyzed for dry matter (**DM**; method 930.15; AOAC, 2019) and nitrogen using the combustion method in a LECO analyzer (method 990.03; AOAC, 2019). Crude protein was calculated as nitrogen × 6.25. Diet and ileal digesta samples were also analyzed for AA (method 982.30 E [a, b, c]; AOAC, 2019), and titanium was quantified

following the method described by Myers et al. (2004). Fecal samples were dried at 55°C in a forced-air oven (Jacobs et al., 2011), finely ground, mixed, and subsampled.

Apparent ileal digestibility (**AID**) and SID of CP and AA were calculated for the four dietary treatments as described by Stein et al. (2007). The values for the basal endogenous losses of CP and AA used to calculate SID were obtained from Adeola et al., (2016).

Subsamples of diets and fecal samples were oven-dried, ground, and analyzed for gross energy (**GE**) to determine apparent total tract digestibility (**ATTD**) using standard bomb calorimetry procedures (Parr 6200, Parr Instruments Company, Moline, IL, USA), and the concentrations of digestible energy (**DE**), ME, and ATTD of GE were calculated using the equations and procedures by Adeola (2001).

### Statistical Analyses

Data was analyzed using the GLIMIX procedure of SAS (SAS Institute Inc., Cary, NC, USA), with pig as the experimental unit. The model included TI level, protease inclusion, and their interaction as fixed effects, while pig and period were included as random effects. Least square means were calculated for each treatment combination, and means were compared using the PDIFF option. Studentized residuals were examined for each observation, and values outside of ±3 standard deviations were considered outliers and removed. Treatment differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

## Results

The effects of SBM source and exogenous protease supplementation on nutrient digestibility were evaluated in growing pigs. The SBM analysis used in this experiment is reported in Table 1. The low TI SBM contained 1.72 TIU/mg, whereas the high TI SBM contained 5.25 TIU/mg. The low TI SBM had lower than expected CP and AA concentrations,

and the high TI SBM was as expected. In addition, the low TI SBM had decreased KOH solubility, PDI, and Lys:CP ratio compared with the high TI SBM. The analyzed nutritional composition of the experimental diets is presented in (**Table 3)**.

There is no evidence of an interaction between protease and SBM source for AID of DM, GE, CP, or most AA (**Table 4**). However, a significant interaction between SBM source and protease supplementation was observed for AID of Cys ($P = 0.022$), and a tendency was observed for Met ($P = 0.089$). Added protease significantly improved the AID of Cys and tended to improve the AID of Met in pigs fed the high TI SBM, whereas pigs fed the low TI SBM showed little to no improvement. For the main effects of the TI concentration in SBM (**Table 5**), pigs fed the high TI SBM had greater ($P < 0.05$) AID of CP, Lys, Asp, Gly, Tyr, and mean dispensable AA and a tendency for greater total AA ($P = 0.057$), compared with pigs fed the low TI SBM. In contrast, pigs fed the low TI SBM had greater AID of Trp ($P = 0.017$) compared with pigs fed the high TI SBM. For the main effect of protease supplementation regardless of SBM source, pigs fed dies with added protease had greater AID of DM, mean indispensable AA, Arg, Ile, Leu, Val, Ala, Asp, Glu, and Ser and tended to have greater ($P < 0.10$) AID of GE, Phe, Thr, and total AA compared with pigs fed diets without protease supplementation.

There is no evidence of an interaction between protease and SBM source for SID of CP and most AA (**Table 6**). However, a significant interaction between SBM and protease was observed for SID of Cys ($P = 0.025$), and tendencies for an interaction ($P < 0.10$) were observed for the SID of Met and Phe. Added protease significantly improved SID of Cys and tended to improve SID of Met in pigs fed the high TI SBM, but had no effect in pigs fed the low TI SBM. In contrast, pigs fed the low TI SBM had the greatest SID of Phe compared with pigs fed the high TI SBM with added protease. However, the magnitude of improvement was greater in pigs

fed the high TI SBM, while pigs fed the low TI SBM showed no improvement. There is no evidence of a main effect of SBM source for the SID of CP and most AA. Pigs fed the low TI SBM had greater ($P < 0.05$) SID of Trp and Phe and tended to have greater SID of Leu ($P = 0.082$) compared with pigs fed the high TI SBM. There is no evidence of a main effect of protease supplementation for the SID of CP (**Table 7**). Regardless of SBM source, pigs fed diets with added protease had greater ($P < 0.05$) SID of Arg, Ile, Leu, Val, Ala, Cys, Glu, Ser, and mean indispensable AA and tended ($P < 0.10$) to have greater SID of Met, Phe, Thr, Asp, and total AA compared with pigs fed diets without protease supplementation.

For the ATTD of DM, CP, or GE (Table 8), there was no evidence of an interaction between protease supplementation and SBM source or main effects for protease or SBM source.

## Discussion

The goal of the experiment was to source a low TI SBM that had less than 3 TIU/mg and a high TI SBM that had more than 5 TIU/mg. The low and high TI SBM achieved the target and was analyzed at 1.72 and 5.25 TIU/mg. Both of these SBM sources were within the expected range of TIU based on previous industry surveys (Gaffield et al. 2024). However, the TIU/mg of low TI SBM was on the low end of the range and the CP and AA content of the low TI SBM was lower than expected, within the lower range of reported values of the NRC (2012). In addition, the low TI SBM had decreased KOH solubility, PDI, and Lys: CP ratio compared with the high TI SBM. The SBM protein solubility in a KOH solution is analyzed to assess the protein solubility, which is an indirect indicator of processing severity and protein quality. Previous research has determined a target KOH protein solubility between 78 and 84%, where values below this range are associated with reduced nitrogen digestibility (Parsons et al., 1991). The low TI SBM had a KOH solubility of 74%, suggesting a decrease in protein availability and

digestibility. Additionally, the Lys: CP ratio in the two SBM sources can also be used as an indirect indicator of processing severity. Previous research determined that the degree of thermal processing may be estimated by calculating the Lys: CP ratio; thermal processing reduces lysine concentration to a greater extent than crude protein (González-Vega et al., 2011). In the current study, the lower values of KOH protein solubility and Lys: CP ratio may indicate greater processing severity of the low TI SBM, which would subsequently result in decreased KOH solubility and TIU. Therefore, it is important to note that the response to TIU is confounded by differences in both processing conditions and inherent soybean batch variability, which likely influence the observed differences in nutrient composition and digestibility.

Pigs fed high TI SBM had greater AID of Lys compared with pigs fed low TI SBM, despite the high TI SBM containing greater concentrations of trypsin inhibitors. However, pigs fed the low TI SBM had greater SID of Trp and Phe and tended to have greater SID of Leu compared with pigs fed the high TI SBM. Since the SBM sources were obtained as commercially processed ingredients, the processing environment or soybean sourcing of each source of SBM cannot be defined. This finding is likely attributed to differences in the degree of thermal processing and resulting protein quality characteristics between SBM sources as reflected by the lower KOH solubility values and the Lys: CP ratio. Previous research indicates that excessive heat treatment reduces Lys digestibility due to the Maillard reaction, whereas insufficient heat treatment results in higher residual TI, impairing proteolysis and reducing protein and AA digestibility (Knabe et al., 1987; González-Vega et al., 2011). Although the low TI SBM had decreased AID Lys compared with the high TI SBM, the AID Lys values were both in agreement with reported values (NRC 2012). When endogenous Lys loss was used to calculate SID Lys, there was no evidence of difference between the two SBM sources. Due to these considerations,

this research conflicts previous research that determined increasing TI concentrations from 2.51 to 8.78 mg/g in soybean meal reduced the AID of CP and AA (Chen et al., 2020). Overall, undefined differences in SBM sources, potential in soybean origin and processing, led to confounding differences in TIU and KOH solubility that resulted in similar SID Lys between SBM sources.

The protease used in this experiment is a serine protease with keratinase activity. A wide range of proteins, including collagen, elastin, keratin, and other protein containing cysteine disulfide bonds, can be broken down by keratinase (Yu et al. 1972, Lin et al., 1996, and Gradisar et al., 2005). Soybean meal contains proteins that are difficult to digest, such as, glycinin, β-conglycinin, trypsin inhibitors, lectins, and other minor proteins (Brandon and Friedman, 2002; Sun et al., 2007; Zhao et al., 2008). Glycinin and β-conglycinin are difficult to digest due to the cystine disulfide bonds; therefore, providing an opportunity for keratinase to improve digestibility (Hou and Chang, 2004; Golubovic et al., 2005). This has been confirmed through in vitro research that demonstrated that keratinase can hydrolyze both soybean glycinin and β-conglycinin and was further validated by improvements in apparent ileal digestibility of AA in pigs fed corn and soybean meal-based diets with added keratinase (Wang et al., 2011).

In our experiment, the proteases improved the SID of most AA in SBM except His, Lys, Trp, Gly, and Tyr. Previous research observed a similar but varying response when adding a protease produced from Bacillus licheniformis PWD-1 to swine diets. Wang et al. (2011) observed an improvement in AID of CP and all AA when adding protease to a corn and soybean meal-based diet that contained 5% wheat midds. In addition, (Huang et al., 2018) reported improvements in most AA when adding a protease to a corn and soybean meal-based diet fed to growing pigs. However, the authors did not observe improvements in SID Lys, His, Gly, and Tyr

similar to the experiment conducted herein. There was also no evidence of differences in SID of Met, Arg, and Ala, which differed from the data reported herein (Huang et al. 2018). In addition, Huang et al. (2018), demonstrated that the effect of added protease on AA digestibility can be influenced by ingredient source. In this experiment, the effects of protease addition were consistent across both SBM sources, except for the SID of Met, Cys, and Phe.

Adding the protease to the high TI SBM improved the ileal digestibility of Met and Cys; however, there was no evidence of difference when adding the protease to the low TI SBM. Previous research has demonstrated an improvement in AID and SID of Cys when protease is added to a corn and soybean-meal based diet; however, the response on SID of Met was inconsistent (Wang et al, 2011; Huang et al., 2018). Huang et al. (2018) demonstrated that adding protease to rice bran, cottonseed meal, and peanut meal improved SID of Met, but not when protease is added to rapeseed meal, corn, and SBM. This led the authors to conclude that the number of disulfide bonds for the protease to break down may influence the response on digestibility (Huang et al., 2018). Although the concentrations of Met and Cys varied between SBM sources, it is unclear what led to the differences in the response to added protease. Previous research investigated the effects of a protease produced by *Bacillus licheniformis* on AA digestibility in broilers fed two different sources of SBM (3.01 mg/g TIA and 85.7% KOH solubility; 5.89 mg/g TIA and 75.5% KOH solubility). In contrast to our experiment, the authors reported no interactions for AID of Met or Cys and improvements in their digestibility with added protease regardless of SBM source.

In addition to improvements in AA digestibility, *Bacillus lichenformis* PWD-1 derived protease has been shown to improve apparent starch digestibility in the distal jejunum and proximal ileum (Selle et al. 2013). Similarly, Chen et al. 2020 reported a tendency for

improvements in AID of GE in pigs fed corn or sorghum and soybean meal-based diets with added protease. This is in agreement with the improvement in AID of GE observed within this experiment. However, there was no evidence of difference observed in ATTD of GE when protease was added to the diet. This can potentially be explained by the microbial fermentation of undigested nutrients in the large intestine.

In conclusion, the SBM source defined as low TI SBM analyzed to contain 1.72 mg/g TI, 43.2% CP, 2.59% lysine, lysine: CP ratio of 5.99%, and KOH solubility of 73.7%. The SBM source is defined as the high TI SBM analyzed to contain 5.25 mg/g TI, 47.9% CP, 3.07% lysine, lysine: CP ratio of 6.4%, and KOH solubility of 82.9%. The high TI SBM resulted in improved AID of CP, Lys, and total AA compared with the low TI SBM. It is hypothesized that this resulted from the high TI SBM still being within acceptable ranges of trypsin inhibitors for SBM. In addition, the reduction in AID of the low TI soybean meal potentially resulted from additional thermal processing that led to the reduction in TI and a reduction in the lysine: CP ratio and KOH solubility. However, when accounting for the endogenous losses, there was no evidence of difference in SID of CP and a majority of the AA. Addition of protease to a semi-purified diet with SBM as the only source of protein resulted in improved AID of GE and AID and SID of a majority of AA regardless of SBM source. However, protease only resulted in improved AID and SID of methionine and cystine in the high TI SBM source.

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## Conflict of interest

The authors declare no conflicts of interest.

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# List of Tables

**Table 2.1. Analyzed composition of soybean meal sources (as – fed basis)[1]**

| Item, % | L-TI SBM[2] | H-TI SBM[3] |
|---|---|---|
| Trypsin inhibitor units (TIU/mg) | 1.72 | 5.25 |
| KOH Solubility, % | 73.7 | 82.9 |
| Protein dispersibility index, % | 14.2 | 18.3 |
| Dry matter, % | 90.7 | 89.6 |
| Crude protein, % | 43.2 | 47.9 |
| Lys:CP ratio, % | 5.99 | 6.40 |
| Indispensable AA, % | | |
| Arginine | 3.03 | 3.57 |
| Histidine | 1.06 | 1.25 |
| Isoleucine | 1.79 | 2.19 |
| Leucine | 3.14 | 3.74 |
| Lysine | 2.59 | 3.07 |
| Methionine | 0.66 | 0.73 |
| Phenylalanine | 1.91 | 2.21 |
| Threonine | 1.71 | 1.98 |
| Tryptophan | 0.48 | 0.57 |
| Valine | 1.56 | 1.91 |
| Dispensable AA, % | | |
| Alanine | 1.87 | 2.16 |
| Aspartic acid | 4.84 | 5.58 |
| Cysteine | 0.62 | 0.72 |
| Glutamic acid | 7.95 | 9.17 |
| Glycine | 1.80 | 2.04 |
| Serine | 2.15 | 2.48 |
| Tyrosine | 1.26 | 1.59 |

[1]All samples were blinded and sent to Novus Analytical Services (St. Charles, MO, USA) for analysis of DM, CP, and AA. Samples were sent to the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO) for analysis of trypsin inhibitor units, KOH solubility, and protein dispersibility index.
[2]Classified as the low trypsin inhibitor soybean meal (1.72 TIU mg/g trypsin inhibitor).
[3]Classified as the high trypsin inhibitor soybean meal (5.25 TIU mg/g trypsin inhibitor).

**Table 2.2 Ingredient and calculated nutrient composition of the diets (as-is basis)**

| Protease: | No | | Added protease | |
|---|---|---|---|---|
| Soybean Meal: | L-TI SBM[1] | H-TI SBM[2] | L-TI SBM | H-TI SBM |
| Ingredients, % | | | | |
| Soybean meal, L-TI | 33.00 | - | 33.00 | - |
| Soybean meal, H-TI | - | 33.00 | - | 33.00 |
| Corn starch | 41.94 | 41.94 | 41.74 | 41.74 |
| Soybean oil | 2.00 | 2.00 | 2.00 | 2.00 |
| Monocalcium phosphate | 1.20 | 1.20 | 1.20 | 1.20 |
| Calcium carbonate | 0.60 | 0.60 | 0.60 | 0.60 |
| Sucrose | 20.00 | 20.00 | 20.00 | 20.00 |
| Sodium chloride | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin premix[3] | 0.125 | 0.125 | 0.125 | 0.125 |
| Trace Mineral premix[4] | 0.125 | 0.125 | 0.125 | 0.125 |
| Phytase[5] | 0.01 | 0.01 | 0.01 | 0.01 |
| Protease[6] | - | - | 0.20 | 0.20 |
| Titanium Dioxide | 0.50 | 0.50 | 0.50 | 0.50 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |

[1]Classified as the low trypsin inhibitor soybean meal (1.72 TIU mg/g trypsin inhibitor).
[2]Classified as the high trypsin inhibitor soybean meal (5.25 TIU mg/g trypsin inhibitor).
[3]Provided per kg of diet: 110 mg of Zn from zinc sulfate; 110 mg of Fe from iron sulfate; 33 mg of Mn from manganese oxide; 17 mg of Cu from copper sulfate; 0.30 mg of Se from sodium selenite; 0.30 mg of I from calcium iodate,
[4]Provided per kg of diet: 4,134 IU vitamin A; 1,653 IU vitamin $D_3$; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin $B_{12}$; 50 mg of niacin; 28 mg pantothenic acid; 8 mg riboflavin.
[5]Quantum Blue 10g (AB Vista, Marlborough, Wiltshire, UK) contained 10,000 FTU/g. At 0.01% inclusion, complete diets were expected to contain 500 FTU/kg with an estimated release of 0.10% STTD P.
[6]Serine protease with keratinase activity (Cibenza DP100, NOVUS International Inc.) was included at 300 U protease activity/g of feed.

**Table 2.3 Analyzed composition of experimental diets (as-fed basis)[1]**

| Protease: | No | | Yes | |
|---|---|---|---|---|
| Soybean meal Source | L-TI SBM[2] | H-TI SBM[3] | L-TI SBM | H-TI SBM |
| Gross energy,[4] kcal/kg | 4566 | 4395 | 4452 | 4526 |
| Dry matter, % | 91.97 | 91.81 | 92.17 | 91.83 |
| Crude protein,[4] % | 13.95 | 16.30 | 14.08 | 16.32 |
| Indispensable AA, % | | | | |
| Arginine | 1.04 | 1.25 | 1.09 | 1.19 |
| Histidine | 0.39 | 0.42 | 0.40 | 0.43 |
| Isoleucine | 0.68 | 0.81 | 0.72 | 0.78 |
| Leucine | 1.14 | 1.35 | 1.21 | 1.30 |
| Lysine | 0.93 | 1.17 | 0.99 | 1.09 |
| Methionine | 0.20 | 0.22 | 0.20 | 0.22 |
| Phenylalanine | 0.75 | 0.89 | 0.78 | 0.86 |
| Threonine | 0.60 | 0.70 | 0.63 | 0.68 |
| Tryptophan | 0.22 | 0.27 | 0.24 | 0.25 |
| Valine | 0.72 | 0.86 | 0.78 | 0.83 |
| Dispensable AA, % | | | | |
| Alanine | 0.65 | 0.77 | 0.71 | 0.75 |
| Aspartic acid | 1.71 | 2.01 | 1.81 | 1.94 |
| Cysteine | 0.19 | 0.21 | 0.19 | 0.21 |
| Glutamic acid | 2.69 | 3.19 | 2.87 | 3.07 |
| Glycine | 0.62 | 0.74 | 0.68 | 0.71 |
| Serine | 0.76 | 0.88 | 0.80 | 0.85 |
| Tyrosine | 0.44 | 0.53 | 0.46 | 0.50 |

[1]Unless otherwise noted, all samples were blinded and sent to Novus Analytical Services (St. Charles, MO, USA) for analysis.
[2]Classified as the low trypsin inhibitor soybean meal (1.72 TIU mg/g trypsin inhibitor).
[3]Classified as the high trypsin inhibitor soybean meal (5.25 TIU mg/g trypsin inhibitor).
[4]Samples were sent to ATC Scientific (North Little Rock, AR, USA) for analysis.

**Table 2.4 Interactive effects of apparent ileal digestibility (AID) of gross energy (GE), dry matter, crude protein, and amino acids (AA) in experimental diets**

| Protease: | No | | Added protease | | | Probability, $P$ = |
|---|---|---|---|---|---|---|
| Soybean meal: | L-TI SBM[1] | H-TI SBM[2] | L-TI SBM | H-TI SBM | SEM | SBM × Protease Interaction[3] |
| AID, % | | | | | | |
| Dry matter | 75.5 | 74.4 | 77.2 | 77.3 | 0.80 | 0.362 |
| Gross energy | 77.7 | 76.4 | 78.1 | 78.6 | 0.97 | 0.194 |
| Crude protein | 75.7 | 78.4 | 76.6 | 79.5 | 1.56 | 0.918 |
| Indispensable AA | | | | | | |
| Arginine | 86.5 | 88.3 | 88.8 | 88.9 | 0.91 | 0.195 |
| Histidine | 88.9 | 88.3 | 89.0 | 89.4 | 0.73 | 0.303 |
| Isoleucine | 78.7 | 79.5 | 80.6 | 80.7 | 1.05 | 0.602 |
| Leucine | 85.3 | 84.5 | 85.9 | 85.8 | 0.78 | 0.483 |
| Lysine | 87.4 | 88.6 | 88.0 | 88.7 | 0.65 | 0.561 |
| Methionine | 84.7 | 84.1 | 84.8 | 85.8 | 0.80 | 0.089 |
| Phenylalanine | 89.6 | 88.5 | 89.7 | 89.9 | 0.65 | 0.134 |
| Threonine | 80.3 | 80.3 | 81.0 | 81.8 | 0.90 | 0.473 |
| Tryptophan | 76.1 | 74.3 | 77.8 | 74.0 | 2.33 | 0.386 |
| Valine | 85.2 | 85.1 | 86.4 | 86.8 | 0.71 | 0.511 |
| Mean | 85.0 | 85.2 | 86.0 | 86.2 | 0.73 | 0.956 |
| Dispensable AA | | | | | | |
| Alanine | 79.0 | 80.1 | 81.3 | 81.7 | 1.23 | 0.602 |
| Aspartic acid | 83.9 | 84.6 | 84.6 | 85.8 | 0.74 | 0.542 |
| Cysteine | 76.5[ab] | 75.3[b] | 76.3[ab] | 78.8[a] | 1.12 | 0.022 |
| Glutamic acid | 87.1 | 86.7 | 87.7 | 88.1 | 0.80 | 0.369 |
| Glycine | 67.8 | 73.7 | 72.9 | 74.1 | 2.67 | 0.157 |
| Serine | 82.7 | 82.7 | 83.6 | 84.2 | 0.84 | 0.599 |
| Tyrosine | 77.0 | 78.8 | 78.0 | 79.9 | 1.28 | 0.943 |
| Mean | 75.9 | 79.8 | 79.2 | 80.7 | 1.90 | 0.325 |
| Total AA | 80.1 | 82.3 | 82.3 | 83.2 | 1.29 | 0.415 |

[a-b]Means within a row lacking a common superscript letter are different ($P < 0.05$) when there is a significant interactive effect.

[1]Classified as the low trypsin inhibitor soybean meal treatment (1.72 TIU mg/g trypsin inhibitor).

[2]Classified as the high trypsin inhibitor soybean meal treatment (5.25 TIU mg/g trypsin inhibitor).

[3]Interactive effect for soybean meal source × protease.

**Table 2.5 Main effects of soybean meal source and protease supplementation on apparent ileal digestibility (AID) of gross energy (GE), dry matter (DM), crude protein (CP), and amino acids (AA) in experimental diets**

| | Soybean meal source | | | | Added protease | | | |
|---|---|---|---|---|---|---|---|---|
| Item, % | L-TI SBM[1] | H-TI SBM[2] | SEM | *P* = | No | Yes | SEM | *P* = |
| AID, % | | | | | | | | |
| Dry matter | 76.4 | 75.8 | 0.68 | 0.374 | 75.0 | 77.3 | 0.68 | 0.001 |
| Gross energy | 77.9 | 78.3 | 0.86 | 0.523 | 77.0 | 77.5 | 0.86 | 0.061 |
| Crude protein | 76.1 | 79.0 | 1.47 | 0.001 | 77.1 | 78.0 | 1.47 | 0.193 |
| Indispensable AA | | | | | | | | |
| Arginine | 87.6 | 88.6 | 0.80 | 0.134 | 87.4 | 88.8 | 0.80 | 0.032 |
| Histidine | 89.0 | 88.7 | 0.66 | 0.843 | 88.6 | 89.2 | 0.66 | 0.152 |
| Isoleucine | 79.7 | 80.1 | 0.94 | 0.478 | 79.1 | 80.7 | 0.94 | 0.023 |
| Leucine | 85.6 | 85.2 | 0.71 | 0.371 | 84.9 | 85.9 | 0.71 | 0.042 |
| Lysine | 87.7 | 88.6 | 0.59 | 0.029 | 88.0 | 88.3 | 0.59 | 0.443 |
| Methionine | 84.8 | 85.0 | 0.73 | 0.716 | 84.4 | 85.3 | 0.73 | 0.065 |
| Phenylalanine | 89.7 | 89.2 | 0.59 | 0.270 | 89.1 | 89.8 | 0.59 | 0.070 |
| Threonine | 80.7 | 81.1 | 0.81 | 0.477 | 80.3 | 81.4 | 0.81 | 0.077 |
| Tryptophan | 77.0 | 74.2 | 2.20 | 0.017 | 75.2 | 75.9 | 2.20 | 0.538 |
| Valine | 85.8 | 85.9 | 0.63 | 0.791 | 85.2 | 86.7 | 0.63 | 0.003 |
| Mean | 85.5 | 85.7 | 0.66 | 0.650 | 85.1 | 86.1 | 0.66 | 0.025 |
| Dispensable AA | | | | | | | | |
| Alanine | 80.2 | 80.9 | 1.14 | 0.280 | 79.6 | 81.5 | 1.14 | 0.008 |
| Aspartic acid | 84.3 | 85.2 | 0.65 | 0.050 | 84.3 | 85.2 | 0.65 | 0.047 |
| Cysteine | 76.4 | 77.1 | 0.98 | 0.387 | 75.9 | 77.5 | 0.98 | 0.044 |
| Glutamic acid | 87.4 | 87.4 | 0.73 | 0.869 | 86.9 | 87.9 | 0.73 | 0.031 |
| Glycine | 70.3 | 73.9 | 2.4 | 0.039 | 70.8 | 73.5 | 0.73 | 0.107 |
| Serine | 83.1 | 83.4 | 0.74 | 0.600 | 82.7 | 83.9 | 0.74 | 0.037 |
| Tyrosine | 77.5 | 79.3 | 1.16 | 0.025 | 77.9 | 78.9 | 1.16 | 0.188 |
| Mean | 77.6 | 80.2 | 1.69 | 0.036 | 77.9 | 79.9 | 1.69 | 0.103 |
| Total AA | 81.2 | 82.8 | 1.16 | 0.057 | 81.2 | 82.8 | 1.16 | 0.057 |

[1]Classified as the low trypsin inhibitor soybean meal treatment (1.72 TIU mg/g trypsin inhibitor).
[2]Classified as the high trypsin inhibitor soybean meal treatment (5.25 TIU mg/g trypsin inhibitor).

**Table 2.6 Interactive effects of standardized ileal digestibility (SID) of crude protein (CP), and amino acids (AA) in experimental diets**

| Protease: | No | | Added protease | | | Probability, $P$ = |
|---|---|---|---|---|---|---|
| Soybean meal: | L-TI SBM[1] | H-TI SBM[2] | L-TI SBM | H-TI SBM | SEM | SBM × Protease Interaction[3] |
| Crude protein | 86.5 | 87.5 | 87.4 | 88.9 | 1.56 | 0.760 |
| Indispensable AA | | | | | | |
| Arginine | 91.7 | 92.6 | 93.7 | 93.4 | 0.91 | 0.324 |
| Histidine | 92.8 | 91.7 | 92.9 | 93.0 | 0.73 | 0.209 |
| Isoleucine | 82.8 | 82.9 | 84.5 | 84.3 | 1.05 | 0.778 |
| Leucine | 89.0 | 87.7 | 89.5 | 89.2 | 0.78 | 0.308 |
| Lysine | 91.0 | 91.5 | 91.4 | 91.7 | 0.65 | 0.914 |
| Methionine | 87.2[ab] | 86.3[b] | 87.3[ab] | 88.1[a] | 0.80 | 0.094 |
| Phenylalanine | 93.1[a] | 91.5[b] | 93.2[a] | 93.0[ab] | 0.65 | 0.082 |
| Threonine | 88.8 | 87.7 | 89.2 | 89.4 | 0.90 | 0.246 |
| Tryptophan | 81.7 | 78.9 | 83.0 | 79.0 | 2.33 | 0.598 |
| Valine | 90.5 | 89.5 | 91.3 | 91.4 | 0.71 | 0.220 |
| Mean | 89.6 | 89.1 | 90.4 | 90.3 | 0.73 | 0.631 |
| Dispensable AA | | | | | | |
| Alanine | 87.7 | 87.5 | 89.4 | 89.2 | 1.23 | 0.992 |
| Aspartic acid | 88.0 | 88.1 | 88.5 | 89.5 | 0.74 | 0.346 |
| Cysteine | 85.2[ab] | 83.2[b] | 85.0[ab] | 86.7[a] | 1.12 | 0.025 |
| Glutamic acid | 90.4 | 89.6 | 90.9 | 91.1 | 0.80 | 0.224 |
| Glycine | 89.3 | 91.9 | 92.8 | 93.0 | 2.67 | 0.480 |
| Serine | 89.3 | 88.5 | 90.0 | 90.2 | 0.84 | 0.350 |
| Tyrosine | 81.9 | 82.9 | 82.7 | 84.2 | 1.28 | 0.739 |
| Mean | 87.6 | 89.8 | 90.3 | 91.0 | 1.90 | 0.533 |
| Total AA | 88.5 | 89.5 | 90.4 | 90.7 | 1.29 | 0.685 |

[a-b]Means within a row lacking a common superscript letter are different ($P < 0.05$) when there is a significant interactive effect.
[1]Classified as the low trypsin inhibitor soybean meal treatment (1.72 TIU mg/g trypsin inhibitor).
[2]Classified as the high trypsin inhibitor soybean meal treatment (5.25 TIU mg/g trypsin inhibitor).
[3]Interactive effect for soybean meal source × protease.

**Table 2.7 Main effects of soybean meal source and protease supplementation on standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in experimental diets**

| | Soybean meal source | | | | Added protease | | | |
|---|---|---|---|---|---|---|---|---|
| Item, % | L-TI SBM[1] | H-TI SBM[2] | SEM | *P* = | No | Yes | SEM | *P* = |
| SID, % | | | | | | | | |
| Crude protein | 87.0 | 88.2 | 1.47 | 0.106 | 87.0 | 88.1 | 1.46 | 0.156 |
| Indispensable AA | | | | | | | | |
| Arginine | 92.7 | 93.0 | 0.80 | 0.611 | 92.2 | 93.6 | 0.80 | 0.029 |
| Histidine | 92.8 | 92.4 | 0.66 | 0.296 | 92.2 | 93.0 | 0.66 | 0.122 |
| Isoleucine | 83.6 | 83.6 | 0.94 | 0.996 | 82.9 | 84.4 | 0.94 | 0.025 |
| Leucine | 89.3 | 88.5 | 0.71 | 0.082 | 88.4 | 89.3 | 0.71 | 0.047 |
| Lysine | 91.2 | 91.6 | 0.59 | 0.318 | 91.3 | 91.6 | 0.59 | 0.419 |
| Methionine | 87.3 | 87.2 | 0.73 | 0.925 | 86.8 | 87.7 | 0.73 | 0.061 |
| Phenylalanine | 93.1 | 92.3 | 0.59 | 0.034 | 92.3 | 93.1 | 0.59 | 0.068 |
| Threonine | 89.0 | 88.5 | 0.81 | 0.418 | 88.2 | 89.3 | 0.81 | 0.089 |
| Tryptophan | 82.3 | 78.9 | 2.20 | 0.005 | 80.3 | 81.0 | 2.20 | 0.549 |
| Valine | 90.9 | 90.5 | 0.63 | 0.304 | 90.0 | 91.3 | 0.63 | 0.005 |
| Mean | 90.0 | 89.7 | 0.66 | 0.423 | 89.3 | 90.3 | 0.66 | 0.026 |
| Dispensable AA | | | | | | | | |
| Alanine | 88.6 | 88.4 | 1.14 | 0.778 | 87.6 | 89.3 | 1.14 | 0.014 |
| Aspartic acid | 88.3 | 88.7 | 0.66 | 0.283 | 88.1 | 89.0 | 0.66 | 0.053 |
| Cysteine | 85.1 | 85.0 | 0.98 | 0.868 | 84.2 | 85.9 | 0.98 | 0.038 |
| Glutamic acid | 90.6 | 90.4 | 0.73 | 0.510 | 90.0 | 91.0 | 0.73 | 0.037 |
| Glycine | 91.0 | 92.5 | 2.39 | 0.393 | 90.6 | 92.9 | 2.39 | 0.175 |
| Serine | 89.7 | 89.3 | 0.74 | 0.548 | 88.9 | 89.3 | 0.74 | 0.041 |
| Tyrosine | 82.5 | 82.3 | 1.16 | 0.126 | 82.4 | 83.5 | 1.16 | 0.171 |
| Mean | 88.9 | 90.7 | 1.69 | 0.241 | 88.7 | 90.7 | 1.69 | 0.118 |
| Total AA | 89.4 | 90.1 | 1.16 | 0.429 | 89.0 | 90.5 | 1.16 | 0.064 |

[1]Classified as the low trypsin inhibitor soybean meal treatment (1.72 TIU mg/g trypsin inhibitor).
[2]Classified as the high trypsin inhibitor soybean meal treatment (5.25 TIU mg/g trypsin inhibitor).

**Table 2.8 Apparent total tract digestibility (ATTD) of dry matter (DM), crude protein (CP), and gross energy (GE) in experimental diets**

| Protease: | No | | Added protease | | | Probability, *P* = | | |
|---|---|---|---|---|---|---|---|---|
| Soybean meal: | L-TI SBM[1] | H-TI SBM[2] | L-TI SBM | H-TI SBM | SEM | SBM | Protease | Interaction[3] |
| ATTD, % | | | | | | | | |
| Dry matter | 91.5 | 91.1 | 91.2 | 91.0 | 0.80 | 0.562 | 0.773 | 0.822 |
| Crude protein | 79.3 | 81.0 | 79.2 | 80.4 | 1.92 | 0.299 | 0.822 | 0.851 |
| Gross energy | 91.2 | 90.9 | 90.7 | 90.8 | 0.83 | 0.833 | 0.657 | 0.729 |

[1]Classified as the low trypsin inhibitor soybean meal treatment (1.72 TIU mg/g trypsin inhibitor).
[2]Classified as the high trypsin inhibitor soybean meal treatment (5.25 TIU mg/g trypsin inhibitor).
[3]Interactive effect for soybean meal source × protease.

# Chapter 3 - Effects of exogenous mannanase, xylanase, and xylanase-glucanase on apparent total tract digestibility of nutrients, metabolizable energy, and N retention in grow-finish pigs

## Lay summary

Corn and SBM (**SBM**) are the most predominant ingredients used in swine diets. Corn is a favorable energy source due to its high starch content and energy density, while SBM is the preferred protein source due to lysine availability being complementary to corn. However, both ingredients contain non-starch polysaccharides (**NSP**) that pigs are unable to digest because they lack endogenous enzymes to break them down. The objective of the study was to determine if adding exogenous carbohydrases individually or in combination to a corn-SBM diet would increase the concentration of apparent total tract digestibility (**ATTD**) of nutrients, apparent metabolizable energy (**AME**), and nitrogen retention in grow-finishing pigs fed corn-SBM diets. Dietary treatments consisted of a corn and SBM-based control diet or the control diet containing β-mannanase, a combination of endo-1,4- β-xylanase & endo-1,4- β – glucanase, a combination of β-mannanase & endo-1,4- β-xylanase & endo-1,4- β – glucanase, or endo-1,4- β-xylanase. The addition of β-mannanase, xylanase & glucanase, and a combination of both increased the Ca digestibility of corn and SBM-based diets fed to grow-finish pigs. Exogenous carbohydrases used in this experiment did not increase the concentration of DE or ME, nitrogen retention, or digestibility of remaining nutrients.

## Teaser text

Exogenous carbohydrases improved the total tract digestibility of calcium, but did not improve energy utilization or apparent total track nitrogen digestibility when fed to grow-finishing pigs.

## Abstract

The objective of this experiment was to compare the effects of carbohydrases on ATTD of nutrients, apparent metabolizable energy, and nitrogen retention in grow-finishing pigs fed corn and SBM-based diets. A total of 10 barrows (average initial BW 55 kg; DNA 200 × 400, DNA Genetics, Columbus, Nebraska, USA) were allotted to a replicated 5 x 4 incomplete Latin square design with 5 dietary treatments and 4 periods of 10-d in each square, resulting in 8 replicate pigs per treatment. Dietary treatments consisted of a basal corn-SBM diet, with the addition of treatments containing β-mannanase (**MN**; 100 g/MT Natupulse; BASF, Florham Park, NJ, USA), a combination of endo-1,4- β-xylanase & endo-1,4- β – glucanase (**XG**; 100 g/MT Natugrain® TS; BASF, Florham Park, NJ, USA), a combination of β-mannanase & endo-1,4- β-xylanase & endo-1,4- β – glucanase (**MXG**; 100 g/MT Natupulse and 100 g/MT Natugrain), or endo-1,4- β-xylanase (**XY**; 20 g/MT Econase XT 5 P; AB Vista, Marlborough, UK). Pigs were housed individually in metabolism crates equipped with a feeder, a drinker, and wire mesh floors that allowed for total and separate collection of feces and urine. There was no evidence of an overall treatment effect on feed intake, GE intake, GE output in urine, ATTD of P, ATTD of ADF, urinary N, and N retention intake. However, there was an overall treatment effect for DE, ME, ATTD of GE, ATTD of DM, ATTD of Ca, and ATTD of NDF. Pigs fed MN, XG, and MXG had greater *(P* < 0.05) ATTD of Ca compared with pigs fed the control diet, with pigs fed XY not differing from other treatments. Consequently, pigs fed the control diet had greater *(P* < 0.05) DE, ME, ATTD of GE, ATTD of DM, and ATTD of NDF compared with pigs fed enzyme-supplemented diets. There was a tendency *(P* < 0.10) for an overall treatment effect for N retained and N digestibility. Pigs fed MN tended to have greater *(P* < 0.10) N retained compared with pigs fed XY, while the control, XG, and MXG did not differ from either

treatment. Consequently, pigs fed the control diet had greater *(P* < 0.05) N digestibility compared with pigs fed MXG, while pigs fed MN, XG, and XY did not differ from either treatment. In conclusion, supplementation of β-mannanase, endo-1,4-β-xylanase and endo-1,4-β-glucanase resulted in the increased ATTD of Ca; however, exogenous carbohydrase supplementation did not improve energy utilization or ATTD of nitrogen when fed to grow-finishing pigs.

**Key words:** mannanase, xylanase, glucanase, energy, nitrogen, pigs

## List of abbreviations:

**ATTD,** apparent total tract digestibility;

**CP,** crude protein;

**DE,** digestible energy;

**DM,** dry matter;

**ME,** metabolizable energy;

**Ca,** calcium;

**P,** phosphorus;

**NDF,** neutral detergent fiber;

**ADF,** acid detergent fiber;

**NSP,** non-starch polysaccharides;

**GE,** gross energy;

**ME,** Metabolizable energy;

**MN,** Mannanase;

**XG,** Xylanase-Glucanase;

**MXG,** Mannanase + Xylanase-Glucanase;

**XY,** Xylanase.

# Introduction

In the United States, corn is the most widely included cereal grain in swine diets due to its energy content, consistent availability, and economic feasibility. Soybean meal (**SBM**) is the predominant protein source included in swine diets due to its high protein content, favorable amino acid (**AA**) profile, consistent availability, and economic feasibility. Despite the routine use of these ingredients, both corn and SBM contain non-starch polysaccharides (**NSP**) that are not degraded due to the lack of endogenous enzymes within the small intestine. Therefore, they reach the large intestine mostly indigested. These NSP consist of cellulose, hemicelluloses, pectic substances, and gums (guar) and mucilages (Caprita et al., 2010).

Corn is the primary energy source in swine diets and is comprised of approximately 83% endosperm, 11% germ, 5% bran, and 1% tip cap, with the bran fraction containing the majority NSP (Watson, 2003; Ai and Jane, 2016). The bran or pericarp fraction contains the majority of NSP despite representing a relatively small proportion of the corn kernel. Arabinoxylans represent the largest fraction of corn NSP found in the pericarp and aleurone layers of corn kernels, followed by cellulose, while β-glucans are present in minimal concentrations. Arabinoxylans consist of a xylose backbone with arabinose substitutions; this structure dictates enzymatic hydrolysis according to the substrate the enzyme can act on.

Soybean meal contains a larger concentration of NSP compared with that of corn, having approximately 21% NSP of DM and 29% being soluble (Bach Knudsen, 1997). The NSP within SBM are categorized as cellulose, non-cellulose polymers and pectic polysaccharides, with the non-cellulosic polymers consisting of arabinoxylans, β-glucans, mannans, galactans, xyloglucan, and fructans (Choct et al., 2010). Previous research has demonstrated that the addition of soluble polysaccharides to a diet decreased the apparent ileal digestibly of nitrogen.  This results from

impaired hydrolysis of protein rather than influencing the absorption of digested products (Murray et al., 1977).

Exogenous carbohydrases are currently available for use in swine diets, including xylanases, β-glucanases, β-mannanases, pectinases, cellulases, and α-galactosidases (Park and Adeola, 2023). Due to the NSP content of cereal grains (e.g. corn, wheat, and barley) used in swine diets, xylanase and β-glucanases have been the predominant exogenous enzymes used (Adeola and Cowieson, 2011). There has also been interest in the use of β-mannanases when feed ingredients contain high concentrations of mannan. Additionally, SBM contains galactomannans, lending to potential improvements when adding β-mannanase to swine diets (Choi et al., 2026).

Recent research demonstrated that nursery pigs fed simple or complex diets with β-mannanase alone or in combination with xylanase, β-glucanase, and arabinofuranosidases had increased apparent total tract digestibility (**ATTD**) of energy and protein (Galli et al., 2024). Therefore, the objective of this study was to compare the effects of β-mannanase, β-1-4-xylanase, a combination of endo-1,4- β-xylanase & endo-1,4- β – glucanase, or a combination of β-mannanase & endo-1,4- β-xylanase & endo-1,4- β – glucanase on ATTD of nutrients, the concentration of metabolizable energy, and nitrogen retention in grow-finishing pigs fed corn and SBM-based diets.

## Materials and methods

### Animal Use

The Institutional Animal Care and Use Committee at Kansas State University approved the protocol used for this experiment. A total of 10 barrows (average initial BW 55.00 kg; DNA 241 × 600, DNA Genetics, Columbus, Nebraska, USA) were housed individually in metabolic

crates (1.52m × 0.84 m) equipped with solid panels on the sides, a feeder, a water cup drinker, and metal diamond-slatted flooring. Each pig was allotted to a replicated 5 x 4 incomplete Latin square design with 5 dietary treatments and 4 periods of 12-d in each square. Therefore, there were 8 replicate pigs per treatment. Each period lasted 12-d, consisting of 5-d, 1d to feed to ferric oxide marker, 5-d of urine, fecal, and orts collection, and 1 d to record the weight of the pigs.

Dietary treatments consisted of a corn and SBM-based control diet or the control diet containing β-mannanase (**MN**; 100 g/MT Natupulse TS; BASF, Florham Park, NJ, USA), a combination of endo-1,4- β-xylanase & endo-1,4- β – glucanase (**XG**; 100 g/MT Natugrain TS; BASF, Florham Park, NJ, USA), a combination of β-mannanase & endo-1,4- β-xylanase & endo-1,4- β – glucanase (**MXG**; 100 g/MT Natupulse TS and 100 g/MT Natugrain TS; BASF, Florham Park, NJ, USA), or endo-1,4- β-xylanase (**XY**; 20 g/MT Econase XT 5 P; AB Vista, Marlborough, UK). A basal corn and SBM-based diet was formulated (**Table 1**) to meet or exceed all nutrient requirements for pigs between 50 and 75 kg (NRC, 2012). Pigs had ad libitum access to water throughout the study and were fed 3.0 times the estimated requirement for maintenance energy (i.e., 197 kcal of ME per kg of $BW^{0.60}$) divided into two equal meals fed at 0800 and 1700 h (Adeola, 2001). Samples of dietary treatments were collected during mixing.

## Sample Collection and Analysis

During the collection period, fecal samples were collected using the marker-to-marker approach described by Adeola (2001). In short, a screen and a urine pan were installed below the floor of the metabolic crates, and an indigestible marker (ferric oxide) was included in the meal fed in the morning of d 6 to signal the start of the collection and in the morning of d 11 to signal the stop of the collection. Fecal samples and orts were collected twice daily during the collection period and each morning a bucket that contained 50mL of 6N HCl to preserve samples, was

placed under the urine pan of each crate. Urine buckets were weighed daily, and the value was recorded prior to taking a subsample of 20% of the total volume of urine in the bucket. The remaining content of the bucket was emptied and HCl was reapplied to the buckets. Fecal, orts, and urine samples were stored at -20°C after collection.

At the conclusion of the experiment, urine samples were thawed and mixed within animal and diet, and a subsample was taken for chemical analyses. Fecal samples were dried in a forced-air oven at 55°C (Jacobs et al., 2011), finely ground, mixed, and subsampled. Fecal, urine, and feed samples were analyzed for DM (Method 930.15; AOAC, 2019), gross energy (**GE**) using bomb calorimetry (Parr 6200, Parr Instruments Company, Moline, IL, USA), N using the combustion method in a LECO analyzer (method 990.03; AOAC, 2019), and crude protein (**CP**) was then calculated as N × 6.25, acid detergent fiber (**ADF**) and neutral detergent fiber (**NDF**) using an ANKOM fiber analyzer (ANKOM technology, Macedon, NY, USA; method 14 and 15), Ca, and P (method 2011.14; AOAC, 2019). In addition, diets were shipped to BASF (Ludwigshafen, Germany) and analyzed for β-mannanase & endo-1,4- β-xylanase & endo-1,4- β – glucanase activity.

Concentrations of digestible energy (**DE**) and ME in each diet were calculated using the following equations:

$$DE_{Diet}, kcal/kg = GE_{Diet} - GE_{Feces} \; ; \; ME_{Diet} = DE_{Diet} - GE_{Urine}$$

where $GE_{Diet}$, $GE_{Feces}$, and $GE_{Urine}$ represent the concentration of energy (kcal/kg) in the feed, fecal samples, and urine samples respectively.

The ATTD of nutrients in each diet was calculated using the following equation:

$$ATTD_{Nutrient}, \% = \left[\left(Nutrient_{intake} - Nutrient_{feces}\right)/Nutrient_{intake}\right] \times 100$$

where $Nutrient_{intake}$ and $Nutrient_{feces}$ represent the daily intake and fecal output (g/d) of the nutrient of interest, respectively.

Lastly, N retention (**Nr**) was calculated using the following equation:

$$Nr, \% = \left[\left(N_{intake} - \left\{N_{feces} + N_{urine}\right\}\right) / N_{intake}\right] \times 100$$

where $N_{intake}$, $N_{feces}$, and $N_{urine}$ represent the daily intake, fecal output, and urine output (g/d) of N, respectively.

### Statistical Analysis

Data were analyzed using the GLIMIX procedure of SAS (SAS Institute Inc., Cary, NC, USA) using pig as the experimental unit. Diet was the fixed effect and pig and period were random effects. Least square means were calculated for each independent variable and means were separated using the PDIFF option. Studentized residuals were calculated for each observation. Observations with a studentized residual outside of ± 3 were considered outliers and removed. Treatment differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

## Results

Analyzed concentrations of GE and nutrients in diet samples (**Table 2**) are within expected ranges, except for the Ca results being greater than expected for the MN, XG, MXG, and XY diets. Enzyme analyses for each diet were within acceptable ranges of expectations.

There was no evidence of differences between dietary treatments for feed intake, GE intake, GE urinary output, apparent total tract digestibility (**ATTD**) of P, and acid detergent fiber (ADF), Urinary N, and N retention (**Table 3**). Diets containing MXG and XY had increased *(*$P < 0.05$) fecal GE output compared with the control diet and MN. This resulted in an overall treatment effect *(*$P < 0.001$) on ATTD of GE and DE and ME, kcal/kg of DM. The ATTD of GE

was lower *(P* < 0.05) in the MXG, XY, and XG diets compared with the control diet. The concentration of DE and ME was greater *(P* < 0.05) in the control diet compared with the XG, MXG, and XY diets. There was an overall treatment effect *(P* < 0.01) for ATTD of DM, NDF, and Ca. However, the ATTD of DM was greater *(P* < 0.05) in the control diet compared with the MXG and XY diets. Additionally, the ATTD of NDF was greater *(P* < 0.05) for the control diet compared with diets containing MN, XG, and XY. However, the ATTD of Ca was greater *(P* < 0.05) in diets containing MN, XG, and MXG compared with the control diet. There was an overall treatment effect *(P* < 0.05) for N intake and fecal N and a tendency *(P* < 0.10) observed for retained N and N digestibility. The N intake was greater *(P* < 0.05) for MXG and XY compared with the MN diet.

## Discussion

Due to the NSP content of cereal grains, e.g. corn, wheat, and barley, and soybean meal used in swine diets, there has been an increased interest in the use of exogenous carbohydrases to improve nutrient digestibility and performance (Adeola and Cowieson, 2011). Exogenous carbohydrases are currently available for use in swine diets, including xylanases, β-glucanases, β-mannanases, pectinases, cellulases, and α-galactosidases (Park and Adeola, 2023). In the present experiment, commercial sources of β-mannanase, β-1-4-xylanase, endo-1,4- β-xylanase and endo-1,4- β – glucanase were applied.

β-mannanans are the NSP that serve as a substrate for β-mannanase. In the diets used within this experiment, soybean meal contains the largest quantity of β-mannanans. β-mannanans are mainly found in the hull fraction of the soybean and they range from 0.7 to 2.1% in soybean meal (Kiarie et al., 2021). Therefore, the amount of hulls remaining in the soybean meal can influence the β-mannanan content. Adding β-mannanase to the diets, containing 16.7% soybean

meal, used in this experiment improved the ATTD of Ca but did not improve the digestibility of energy or remaining nutrients. This agrees with Pettey et al. (2002) who observed no improvements in ATTD of energy, nitrogen, or phosphorus when β-mannanase was added to a corn and SBM (14.4% SBM)-based diet fed to finishing pigs. In contrast, Lv et al. (2013) determined that adding β-mannanase to a diet containing 22.6% SBM and 5.0% wheat midds improved the digestibility of energy and other nutrients.

Previous research has also reported an improvement in ATTD of Ca and P when β-mannanase was added to the diet (Lv et al., 2013). This was similar to the improvement in ATTD of Ca observed in the experiment reported herein; however, there was no difference in the ATTD of P. Overall, the ATTD Ca and P coefficients were greater in the experiment reported herein due to the addition of Phytase to diet. This could potentially explain the difference in the response to β-mannanase on ATTD of P, although there were still improvements in ATTD of Ca.

Corn NSP consist of arabinoxylans, β-glucans, and cellulose, with arabinoxylan making up nearly half of the NSP (Englyst et al. 1982). Corn and soybean meal-based diets typically contain approximately 2.3% to 3.8% of arabinoxylans and 0.6% to 2.1% of β-glucans (Knudsen, 1997; Jaworski et al., 2015; Mathlouthi et al., 2002; Sampson et al., 2015; Yu et al., 2018). Both xylans and β-glucans increase digesta viscosity resulting in reduced nutrient digestibility in feeds. In addition, an increase in undigested nutrients in the intestines can support the proliferation of harmful microbiota (Agyekum and Nyachoti, 2017).

Xylans are the target substrate of xylanase, with xylanase hydrolyzing the β -1, 4-glycosidic bonds of arabinoxylans. Therefore, adding exogenous xylanase to swine diets, provide the potential to improve the utilization of arabinoxylans in corn (Gutierrez et al., 2014).

Previous research has demonstrated improvements in in the AID of NSP and arabinoxylan in pigs fed various sources of xylanase (Ndou et al., 2015).

Corn NSP also consist of β-glucans, which can be hydrolyzed into glucooligosaccharides by β-glucanase (Li et al., 1996). However, the concentration β-glucans in the diet can influence the response β-glucanase has on energy and nutrient digestibility. Li et al. (1996) determined that addition of β-glucanase to a barley and soybean meal-based diet, high in β-glucans, improved the digestibility of energy, crude protein, and amino acids. However, β-glucanase did not influence energy or nutrient digestibility in a wheat and soybean meal-based diet. However, the ileal digestibility of β-glucans was improved in both barley and wheat-based diets.

Commercial applications of xylanase are available individually or in combination with β-glucanase for use in swine diets. Adding β-1-4-xylanase alone or adding endo-1,4- β-xylanase and endo-1,4- β – glucanase in combination to grow-finish pig diets used in this experiment improved the ATTD of Ca but did not improve the digestibility of energy or remaining nutrients. Torres-Pitarch et al. (2019), conducted a meta-analysis and determined addition of xylanase to the diet, regardless of diet type, improved ATTD of DM, GE, and CP, which does not agree with the data reported herein. However, Petry and Patience (2000) conducted a review that determined 50% of the literature found that xylanase improved ATTD of fiber. Additional research has been conducted to determine if adding β – glucanase to diets containing xylanase would further improve nutrient digestibility. The authors determined that increasing the level of β – glucanase in the diets did not further reduce digesta viscosity. However, increasing β – glucanase tended to improve the ATTD of GE; however, there was no differences in the ATTD of DM or CP (Duarte et al., 2021). The data reported herein, demonstrated no differences in the

response to xylanase with or without β – glucanase; however, it is important to note that the sources of xylanase used were different.

In conclusion, the addition of β-mannanase, a combination of endo-1,4- β-xylanase & endo-1,4- β – glucanase, and a combination of both increased the Ca digestibility of corn and soybean meal-based diets fed to grow-finish pigs. Exogenous carbohydrases used in this experiment did not result in improvements DE or ME, nitrogen retention, or digestibility of remaining nutrients.

## Conflict of interest

The authors declare no conflicts of interest.

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# List of tables

**Table 3.1 Ingredient and calculated nutrient composition of experimental diet**

| Item, % | Control | MN[4] | XG[5] | MXG[6] | XY[7] |
|---|---|---|---|---|---|
| Corn | 79.85 | 79.84 | 79.84 | 79.83 | 79.85 |
| SBM, dehull, sol ext. | 16.70 | 16.70 | 16.70 | 16.70 | 16.70 |
| Monocalcium phosphate | 0.95 | 0.95 | 0.95 | 0.95 | 0.95 |
| Calcium carbonate | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Sodium chloride | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| L-Lys HCl | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| DL-Met | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 |
| L-Thr | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| L-Trp | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| Mineral premix[1] | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Vitamin premix[2] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Phytase[3] | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 |
| Mannanase[4] | - | 0.01 | - | 0.01 | - |
| Xylanase + Glucanase[5] | - | - | 0.01 | 0.01 | - |
| Xylanase[6] | - | - | - | - | 0.002 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Calculated composition | | | | | |
| Gross energy, kcal/kg | 3,879 | 3,879 | 3,879 | 3,879 | 3,879 |
| Metabolizable energy, kcal/kg | 3,287 | 3,287 | 3,287 | 3,287 | 3,287 |
| Crude protein, % | 14.61 | 14.61 | 14.61 | 14.61 | 14.61 |
| Crude fat, % | 3.03 | 3.03 | 3.03 | 3.03 | 3.03 |
| Crude fiber, % | 2.23 | 2.23 | 2.23 | 2.23 | 2.23 |
| NDF, % | 8.64 | 8.64 | 8.64 | 8.64 | 8.64 |
| ADF, % | 3.18 | 3.18 | 3.18 | 3.18 | 3.18 |
| Ca, % | 0.63 | 0.63 | 0.63 | 0.63 | 0.63 |
| P, % | 0.53 | 0.53 | 0.53 | 0.53 | 0.53 |

[1]Provided the following quantities of minerals per kg of diet: Copper 11,023 mg, Iodine 198.4 mg, Iron 73,414 mg, Manganese 22,046 mg, Selenium 198.4 mg, Zinc 73,414.

[2]Provided the following quantities of vitamins per kg of diet: Vitamin A 1,653,439 IU, Vitamin D3 661,376 IU, Vitamin E 17,637 IU, Vitamin B12 13.2 mg, menadione 1,323 mg, riboflavin 3,307 mg, d-Pantothenic Acid 11,023 mg, niacin 19,841 mg.

[3]Natuphos 1000 G, BASF, Mt. Olive, NY, USA.

[4]MN, β-mannanase; 100 g/MT Natupulse TS, BASF, Mt. Olive, NY, USA.

[5]XG, combination of endo-1,4- β-xylanase & endo-1,4- β – glucanase; 100 g/MT Natugrain® TS; BASF, Mt. Olive, NY.

[6]MXG, combination of β-mannanase, endo-1,4- β-xylanase & endo-1,4- β – glucanase; 100 g/MT Natupulse TS, BASF, Mt. Olive, NY, USA & 100 g/MT Natugrain® TS; BASF, Mt. Olive, NY.

[7]XY, endo-1,4- β-xylanase; 20 g/MT Econase XT 5 P; AB Vista, Marlborough, UK.

**Table 3.2 Analyzed composition of experimental diets (as-fed basis)[1]**

| Item, | Control | MN[2] | XG[3] | MXG[4] | XY[5] |
|---|---|---|---|---|---|
| Dry matter, % | 87.81 | 87.73 | 87.76 | 87.75 | 87.76 |
| Gross energy, kcal/kg | 3,880 | 3,889 | 3,840 | 3,828 | 3,876 |
| Crude protein, % | 14.92 | 14.65 | 15.49 | 15.24 | 15.11 |
| NDF, %[1] | 10.70 | 9.10 | 10.10 | 10.20 | 10.00 |
| ADF, %[2] | 4.10 | 4.80 | 4.90 | 4.80 | 4.80 |
| Calcium, % | 0.67 | 0.90 | 0.92 | 0.88 | 0.80 |
| Phosphorus, % | 0.50 | 0.52 | 0.54 | 0.54 | 0.60 |
| Natuphos, FTU/kg | 630 | 630 | 650 | 510 | 570 |
| Natugrain, TXU/kg | - | - | 580 | 600 | - |
| Natupulse, TMU/kg | - | 980 | - | 880 | - |
| Econase, TXU/kg | - | - | - | - | - |

[1]Samples were analyzed for nutrients at ATC Scientific in North Little Rock, AR, USA.

Samples were analyzed for enzyme activity at BASF, Ludwigshafen, Germany.

[2]MN, β-mannanase; 100 g/MT Natupulse TS, BASF, Mt. Olive, NY, USA.

[3]XG, combination of endo-1,4- β-xylanase & endo-1,4- β – glucanase; 100 g/MT Natugrain® TS; BASF, Mt. Olive, NY.

[4]MXG, combination of β-mannanase, endo-1,4- β-xylanase & endo-1,4- β – glucanase; 100 g/MT Natupulse TS, BASF, Mt. Olive, NY, USA & 100 g/MT Natugrain® TS; BASF, Mt. Olive, NY.

[5]XY, endo-1,4- β-xylanase; 20 g/MT Econase XT 5 P; AB Vista, Marlborough, UK.

**Table 3.3 Concentrations of digestible energy (DE) and metabolizable energy (ME), and apparent total tract digestibility (ATTD) of gross energy (GE), dry matter (DM), calcium (Ca), phosphorus (P), neutral detergent fiber (NDF), and acid detergent fiber (ADF) in corn and SBM-based control diets and diets with exogenous enzymes[1]**

| Item, | Control | MN[2] | XG[3] | MXG[4] | XY[5] | SEM | *P* < |
|---|---|---|---|---|---|---|---|
| Feed intake, kg/d | 2.38 | 2.24 | 2.38 | 2.47 | 2.49 | 0.210 | 0.118 |
| GE Intake, kcal/d | 9,246 | 8,726 | 9,138 | 9,463 | 9,830 | 841.9 | 0.115 |
| GE Output feces kcal/d | 1,104$^{b}$ | 1,098$^{b}$ | 1,188$^{ab}$ | 1,269$^{a}$ | 1,308$^{a}$ | 80.2 | 0.002 |
| GE Output urine kcal/d | 124 | 123 | 116 | 150 | 131 | 18.5 | 0.572 |
| DE, kcal/kg of DM | 3,889$^{a}$ | 3,870$^{ab}$ | 3,798$^{cd}$ | 3,764$^{d}$ | 3,819$^{bc}$ | 31.5 | 0.001 |
| ME, kcal/kg of DM | 3,829$^{a}$ | 3,807$^{ab}$ | 3,741$^{c}$ | 3,695$^{c}$ | 3,754$^{bc}$ | 34.0 | 0.001 |
| ATTD of GE, % | 88.0$^{a}$ | 87.3$^{ab}$ | 86.8$^{b}$ | 86.3$^{b}$ | 86.5$^{b}$ | 0.72 | 0.004 |
| ATTD of DM, % | 90.6$^{a}$ | 90.0$^{ab}$ | 89.6$^{ab}$ | 89.2$^{b}$ | 89.3$^{b}$ | 0.52 | 0.003 |
| ATTD of Ca, % | 62.0$^{b}$ | 70.0$^{a}$ | 70.8$^{a}$ | 69.3$^{a}$ | 65.4$^{ab}$ | 2.52 | 0.006 |
| ATTD of P, % | 49.1 | 48.0 | 50.5 | 49.9 | 53.8 | 3.78 | 0.616 |
| ATTD of NDF, % | 75.3$^{a}$ | 69.7$^{bc}$ | 68.0$^{cd}$ | 73.4$^{ab}$ | 64.7$^{d}$ | 1.94 | 0.001 |
| ATTD of ADF, % | 78.4 | 79.6 | 78.9 | 77.5 | 76.6 | 1.41 | 0.297 |

$^{a-c}$Values within a row lacking a common superscript letter are different ($P < 0.05$).

[1]A total of 10 barrows (DNA 200 × 400, DNA; initial body weight 55kg) were used in this experiment. Each pig was allotted to a 5 × 4 replicated incomplete Latin square design with 5 dietary treatments and 4 periods for a total of 8 replicate pigs per treatment.

[2]MN, β-mannanase; 100 g/MT Natupulse TS, BASF, Mt. Olive, NY, USA.

[3]XG, combination of endo-1,4- β-xylanase & endo-1,4- β – glucanase; 100 g/MT Natugrain® TS; BASF, Mt. Olive, NY.

[4]MXG, combination of β-mannanase, endo-1,4- β-xylanase & endo-1,4- β – glucanase; 100 g/MT Natupulse TS, BASF, Mt. Olive, NY, USA & 100 g/MT Natugrain® TS; BASF, Mt. Olive, NY.

[5]XY, endo-1,4- β-xylanase; 20 g/MT Econase XT 5 P; AB Vista, Marlborough, UK.

**Table 3.4 Nitrogen balance of pigs fed corn and SBM-based control diets and diets with exogenous enzymes[1]**

| Item, | Control | MN[2] | XG[3] | MXG[4] | XY[5] | SEM | *P*-value |
|---|---|---|---|---|---|---|---|
| N intake, g/d[5] | 56.90[ab] | 52.53[b] | 58.96[ab] | 60.26[a] | 60.21[a] | 5.131 | 0.013 |
| Fecal N, g/d[6] | 7.10[y] | 7.06[y] | 7.53[xy] | 8.58[x] | 8.09[xy] | 0.488 | 0.040 |
| Urinary N, g/d[7] | 11.29 | 11.36 | 11.10 | 12.48 | 11.13 | 2.32 | 0.920 |
| N retained, g/d[8] | 38.57[xy] | 34.17[x] | 40.00[xy] | 39.30[xy] | 41.02[y] | 3.256 | 0.079 |
| N retention, % of intake[9] | 68.29 | 65.03 | 68.40 | 65.50 | 67.94 | 2.423 | 0.601 |
| N digestibility, %[10] | 87.42[x] | 86.42[xy] | 86.91[xy] | 85.38[y] | 86.39[xy] | 0.923 | 0.096 |

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

[x-z]Means within a row lacking a common superscript letter are different ($P < 0.10$).

[1]A total of 10 barrows (DNA 200 × 400, DNA; initial body weight 55kg) were used in this experiment. Each pig was allotted to a 5 × 4 replicated incomplete Latin square design with 5 dietary treatments and 4 periods for a total of 8 replicate pigs per treatment.

[2]MN, β-mannanase; 100 g/MT Natupulse TS, BASF, Mt. Olive, NY, USA.

[3]XG, combination of endo-1,4- β-xylanase & endo-1,4- β – glucanase; 100 g/MT Natugrain® TS; BASF, Mt. Olive, NY.

[4]MXG, combination of β-mannanase, endo-1,4- β-xylanase & endo-1,4- β – glucanase; 100 g/MT Natupulse TS, BASF, Mt. Olive, NY, USA & 100 g/MT Natugrain® TS; BASF, Mt. Olive, NY.

[5]XY, endo-1,4- β-xylanase; 20 g/MT Econase XT 5 P; AB Vista, Marlborough, UK.

[6]N intake, g/d = feed intake during collection period, g/d × (feed N content, % ÷ 100)

[7]Fecal N, g/d = feces weight, g/d, air-dry basis × (feces N content, % ÷ 100)

[8]Urinary N, g/d = urine weight, g/d × (urine N content, % ÷ 100)

[9]N retained, g/d = N intake, g/d – Fecal N, g/d – Urinary N, g/d

[9]N retention, % of intake = (N retained g/d ÷ N intake, g/d) × 100

[10]N digestibility, % = Apparent total tract digestibility of N; ((N intake, g/d – Fecal N, g/d) ÷ N intake, g/d) × 100.