Effects of SID Lys:CP ratios, specialty protein products, and hydrolyzed yeast on nursery pig growth performance, fecal characteristics, and antioxidant status

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# Abstract

This thesis contains three chapters that includes: 1) evaluating the effects of standardized ileal digestible lysine to crude protein (SID Lys:CP) ratios on growth performance of 11- to 25-kg pigs, 2) evaluating the effects of novel specialty soy protein products on growth performance and fecal dry matter (DM) of nursery pigs, and 3) evaluating the effects of hydrolyzed yeast on weanling pig growth performance, fecal characteristics, and stress-related blood antioxidant criteria. Chapter 1 utilized 10,207 nursery pigs in three experiments to determine whether nitrogen (N) is the limiting factor in low protein, amino acid fortified diets, and to evaluate the effects of SID Lys:CP ratios of 11- to 25-kg pigs. Diets formulated to a SID Lys:CP ratio greater than approximately 6.5% decreased feed efficiency (G:F) but adding a N source to low protein diets formulated above this ratio improved G:F. Chapter 2 involved 1,614 pigs in two experiments to evaluate the efficacy of novel specialty soy protein products (soy protein concentrate and thermo-mechanically processed soybean meal) on growth performance and fecal DM of nursery pigs. Ultimately, replacing 25 to 50% of the digestible Lys provided by soybean meal in diets for 5 to 13 kg pigs with specialty soy products improved growth performance, while total replacement resulted in decreased performance. Chapter 3 utilized 360 weanling pigs to evaluate the efficacy of a novel hydrolyzed yeast (HY) product on growth performance, fecal DM, stress-related blood antioxidant criteria, and cytokine production. Overall, feeding 0.04% HY improved G:F. Increasing HY inclusion had no impact on growth performance, but improved early nursery fecal DM. The addition of 0.04% HY with carbadox tended to reduce serum cytokine concentrations. In summary, these experiments provide data on the optimal SID Lys:CP ratio for 11- to 25-kg pigs, the effects of novel soy protein products in nursery pig diets,

and the effects of hydrolyzed yeast on nursery pig growth performance, fecal characteristics, stress-related blood antioxidant criteria, and cytokine parameters.

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# Chapter 1 - Effects of standardized ileal lysine:crude protein ratio and the use of non-protein nitrogen on growth performance of 11- to 25-kg pigs

## Abstract

Three experiments were conducted to determine if nitrogen is a limiting factor for growth performance when feeding low protein, amino acid (AA) fortified diets and determine the effects of standardized ileal digestible lysine to crude protein (SID Lys:CP) ratio on growth performance of 11- to 25-kg pigs. In Exp. 1, 981 pigs ([Fast Large White × PIC L02] × PIC 800; initially 10.3 ± 0.19 kg) were used in a 21-d study. Diets were corn-soybean meal-based consisting of: 1) a low level of feed-grade AA with a SID Lys:CP ratio of 6.0%; 2) a moderate level of feed-grade AA with a SID Lys:CP ratio of 6.5%; 3) a high level of feed-grade AA with a SID Lys:CP ratio of 7.0%; 4) diet 3 with added diammonium phosphate (DAP) added to achieve a SID Lys:CP ratio of 6.5%; and 5) diet 3 with L-Gly added to achieve a SID Lys:CP ratio of 6.5%. Average daily gain (ADG) was unaffected by dietary treatment but gain:feed ratio (G:F) decreased (linear, $P = 0.002$; quadratic, $P = 0.054$) as SID Lys:CP ratio exceeded 6.5%. Adding DAP or L-Gly to the high feed-grade AA diet increased ($P \leq 0.003$) G:F compared to pigs fed the high feed-grade AA diet. In Exp. 2, 4,167 pigs (337 × 1050, PIC; initially 13.0 ± 0.27 kg) were used in a 14-d study. Treatments were arranged in a 2 × 5 factorial with main effects of SID Lys (1.15 or 1.30%) and SID Lys:CP ratio (6.00, 6.22, 6.46, 6.72, and 7.00%). Overall ADG was unaffected by dietary treatment; however, a SID Lys:CP × SID Lys interaction was observed for G:F (linear, $P = 0.012$) where increasing SID Lys:CP ratio decreased (linear, $P < 0.001$) G:F at both SID Lys levels with a more pronounced effect in diets formulated to 1.15% SID Lys. Lastly, Exp. 3 used 5,059 pigs (PIC 800 × Camborough and DNA 600 × 241; initially 11.0 ± 0.90 kg) in an 18-d trial. Treatment diets were arranged in a 2 × 6 factorial with main effects of dried distillers grains with solubles (DDGS; 0 or 15%) and SID Lys:CP ratio (6.01, 6.22, 6.45, 6.70, 6.97, and 7.26%). Overall, ADG was unaffected by dietary treatment, but a SID Lys:CP × DDGS interaction was observed (linear, $P < 0.001$) where G:F increased then decreased (quadratic, $P < 0.001$) in diets without DDGS, whereas in the diets with DDGS, G:F decreased (linear and quadratic, $P \leq 0.002$) as SID Lys:CP ratio increased above 6.45%. In summary, a SID Lys:CP ratio greater than approximately 6.5% decreased G:F, but adding a protein or non-protein nitrogen source to low protein diets formulated above this ratio improves G:F.

**Key Words:** crude protein, amino acids, non-protein nitrogen, nursery pigs, lysine

## Introduction

Although crude protein (CP) is a fundamental component of swine diets, the primary nutritional focus is on supplying adequate essential amino acids (EAA) and sufficient nitrogen (N) to support the endogenous synthesis of non-essential amino acids (NEAA). Because Lys is the first limiting amino acid (AA) in typical swine diets, it is present in the lowest amount relative to the pig's requirement. Therefore, the requirements for other EAA can be expressed relative to Lys (Boisen et al., 2000). Hence, an ideal protein profile can be used to formulate diets that meet the pig's requirements with minimal excess (Zhao et al., 2019; Yang et al., 2020). This concept has been expanded to include an optimal balance between EAA and NEAA (Wang and Fuller, 1989; Lenis et al., 1999). As the dietary CP level decreases, the supply of N available for endogenous NEAA synthesis also declines; therefore, when formulating low protein, AA fortified diets, a NEAA-N, or a requirement for total N should be considered (Boisen, 1997).

Based on empirical data, the NRC (2012) estimates a standardized ileal digestible (SID) Lys requirement of 1.23% and total dietary N of 3.02% for pigs weighing 11 to 25 kg. These values imply that diets should be formulated to maintain a SID Lys:CP ratio at or below 6.5% for 11- to 25-kg pigs. Maintaining the ratio below this threshold ensures that the diet contains sufficient intact protein to supply N for endogenous NEAA synthesis. However, excess CP in the diet may lead to excess AA which must be deaminated and excreted in urine (van Milgen and Dourmad, 2015; Wang et al., 2018). Conversely, if the SID Lys:CP ratio is too high, the diet lacks sufficient N for the synthesis of NEAA (Gloaguen et al., 2014).

The use of feed-grade AA in low protein diets has greatly benefited the swine industry by lowering feed costs, reducing N excretion, and minimizing swine production's environmental impact. Diets high in CP can increase the amount of undigested proteins in the large intestine, which may promote the proliferation of pathogenic bacteria and cause digestive issues. As a result, partially replacing intact protein sources, like SBM, with feed-grade AA can help maintain or even enhance pig performance, particularly feed efficiency (Gloaguen et al., 2014). However, Gotterbarm et al. (1998) and Heger et al. (1998) observed that growth performance may be decreased due to low dietary CP despite meeting EAA requirements. This is thought to be because of decreasing NEAA nitrogen (NEAA-N) to total N ratio. When dietary NEAA-N is insufficient, the required N may come from the catabolism of EAA, reducing the efficiency of EAA utilization for lean growth (Buchinski et al., 2024a). If the dietary CP is inadequate, a N source may be provided in the diet to provide sufficient N for the synthesis of NEAA. This concept is supported by research conducted by Mansilla et al. (2017) and Buchinski et al.

(2024b) who observed that ammonia can serve as a non-protein nitrogen (NPN) source when N is limiting in diets to recover this loss in growth performance.

Although a SID Lys:CP ratio of 6.5% for 11- to 25-kg pigs can be implied from the NRC (2012), further research is needed to validate this ratio. Therefore, the objectives of these three experiments utilizing 11- to 25-kg pigs were 1) to determine whether N is a limiting factor on growth performance when feeding low protein, AA fortified diets, and 2) to determine the optimal SID Lys:CP ratio in diets containing different inclusions of SBM. It was hypothesized that under-feeding N would negatively affect growth performance and that a balance between Lys and CP is necessary when feeding low protein, AA fortified diets.

## Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments (IACUC #4892, #5008, #4996). All experiments were conducted at commercial research sites and pigs were provided ad libitum access to feed and water. Experiment 1 was conducted in southwest Minnesota (New Fashion Pork, Round Lake, MN). The facility contained 52 pens and was completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contained a three-hole, dry self-feeder and a bowl waterer. Experiment 2 was conducted in north central Ohio (Hord Family Farms, Bucyrus, OH). A total of 160 pens were used with 80 double-sided, five-hole stainless steel fence-line feeders, each feeding two adjacent pens. Each pen was also equipped with a cup waterer. Experiment 3 was conducted in southwest Minnesota (Hubbard Feeds, Sleepy Eye, MN). The barns were mechanically ventilated with totally slatted floors. A total of 143 pens were used, each equipped with a five-hole stainless steel dry self-feeder and a bowl waterer.

## Experiment 1

### Animal and Treatment Structure

The objective of Exp. 1 was to determine if N is a limiting factor to maintain feed efficiency when feeding low protein, AA fortified diets. A total of 981 pigs ([Fast Large White × PIC L02] × PIC 800; initially 10.3 ± 0.19 kg) were used in a 21-d growth study. Pigs were housed in mixed-sex pens and pens were allotted to one of five dietary treatments in a randomized complete block design with initial weight as a blocking factor. There were 19 to 20 pigs per pen and 10 pens per treatment. Prior to phase 3 diet formulation, composite corn and soybean (SBM) samples were submitted to Market 1, AgState (Cherokee, IA) for proximate analysis and AA profile using NIR (Table 1). Treatment diets were corn-SBM-based and consisted of: 1) a low level of feed-grade AA with a SID Lys:CP ratio of 6.0%; 2) a moderate level of feed-grade AA with a SID Lys:CP ratio of 6.5%; 3) a high level of feed-grade AA with a SID Lys:CP ratio of 7.0%; 4) diet 3 with added diammonium phosphate (DAP) to achieve a SID Lys:CP ratio of 6.5%; and 5) diet 3 with added L-Gly to achieve a SID Lys:CP ratio of 6.5% (Table 2). Feed-grade AA replaced SBM in the diets to form the first three dietary treatments. Dietary additions of feed-grade AA were adjusted to meet or exceed NRC (2012) AA requirement estimates in relation to Lys for Met and Cys, Thr, Trp, Val, Ile, and His. Treatment diets were manufactured at the New Fashion Pork feed mill (Round Lake, MN). Titanium dioxide was included at 0.4% in the diets as an indigestible marker to determine apparent total tract digestibility (ATTD) of DM and CP. Diet samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratory for CP (Method 990.03; AOAC International, 2006), ash, dry matter (DM; Method 935.29; AOAC International, 2006), ether extract (ANKOM Technology, 2004), crude fiber (ANKOM Technology, 2005), and complete AA profile (Method 982.30; AOAC International, 2006; Table 3).

Pens of pigs were weighed and feed disappearance was measured at the beginning and end of the study (d 21) to determine average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F). Daily feed additions were recorded with a computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN). Blood samples were collected from unfasted pigs on d 21 from four pigs per pen (two barrows and two gilts of medium size) to measure serum blood urea nitrogen (BUN; DetectX Urea Nitrogen Detection Kit; Arbor Assays, Ann Arbor, MI).

**Digestibility Analysis**

Fecal samples were collected on d 21 from three pigs per pen to determine percentage DM. Samples were dried at 55°C in a forced-air oven for 48 h, and the ratio of dried-to-wet fecal weight determined the fecal DM. Fecal samples were analyzed separately for each pig and the average of the three samples from each pen was then used for statistical analysis. Following fecal DM determination, both ground feed and pen-level pooled fecal samples were dried in a 135°C oven for 2 h to determine percentage DM of the samples used for titanium analysis (Method 985.01; AOAC, 2007). Fecal samples were analyzed for DM, N, and $TiO_2$ at the Kansas State University Swine Laboratory. Titanium dioxide concentration in both dried feed and fecal samples were determined using procedures outlined by Leone (1973). The ATTD of DM and N was calculated using the index method described by Adeola (2001) using the following equation:

$$\text{ATTD DM or N, \%} = \left[1 - \left[\left(\frac{\text{DM or N, \%}_{\text{Fecals}}}{\text{DM or N, \%}_{\text{Feed}}}\right) \times \left(\frac{\text{TiO}_2\text{, \%}_{\text{Feed}}}{\text{TiO}_2\text{, \%}_{\text{Fecals}}}\right)\right]\right] \times 100$$

## Experiment 2

The objective of Exp. 2 was to evaluate SID Lys:CP ratios in diets formulated to different levels of SID Lys, and thus different dietary SBM inclusion levels. A total of 4,167 pigs (337 × 1050, PIC; initially 13.0 ± 0.27 kg) originating from three sow farms were placed in the research facility over a 6-d period and used in a 14-d study. Pigs were housed in same-sex pens and pens were allotted to one of ten dietary treatments in a randomized complete block design with blocking structure including sow farm origin, date of entry into the nursery facility, and average pen BW. Each feeder (considered the experimental unit) provided feed for one pen of 26 gilts and one pen of 26 barrows. Composite samples of corn and SBM were analyzed prior to diet formulation at the University of Missouri Agricultural Experiment Station Chemical Laboratory for proximate analysis and complete AA profile (Table 4). Diets were corn-SBM-based and arranged in a 2 × 5 factorial with main effects of SID Lys (1.15 or 1.30% SID Lys) and SID Lys:CP ratio (6.00, 6.22, 6.46, 6.72, and 7.00%) with eight replications per dietary treatment (Table 5). Dietary additions of feed-grade AA were adjusted to meet or exceed NRC (2012) AA requirement estimates in relation to Lys for Met and Cys, Thr, Trp, Val, Ile, and His. Both low and high SID Lys:CP ratio diets, 6.00 and 7.00%, respectively, with low and high SID Lys, were manufactured at the Hord Elevator (Bucyrus, OH) in meal form and blended at the farm by an electronic feeding system (Dry Extract; Big Dutchman, Inc., Holland, MI) to make intermediate dietary treatments. Diet samples were collected and analyzed for proximate analysis and complete AA profile at the University of Missouri Agricultural Experiment Station Chemical Laboratory (Table 6).

Pens of pigs were weighed and feed disappearance was measured at the beginning and end of the study (d 14) to determine ADG, ADFI, and G:F. Daily feed additions were recorded by the electronic feeding system.

## Experiment 3

The objective of Exp. 3 was to evaluate SID Lys:CP ratios in diets containing different basal levels of SBM. This was completed by using 15% DDGS to create high and low levels of SBM within the differing SID Lys:CP ratios. A total of 5,059 (PIC 800 × Camborough and DNA 600 × 241; initially 11.0 ± 0.90 kg) originating from two sow farms were placed in two rooms at the research facility and used in an 18-d study. Pigs were housed in mixed-sex pens with approximately 35 pigs per pen, and pens of pigs were allotted to 1 of 12 dietary treatments in a completely randomized design when pigs reached approximately 11 kg. Composite samples of corn, SBM, and DDGS were collected prior to diet formulation and analyzed for proximate analysis and complete AA profile at the University of Missouri Agricultural Experiment Station Chemical Laboratory (Table 7). Treatment diets were corn-SBM-based and arranged in a 2 × 6 factorial with main effects of DDGS (0 or 15%) and SID Lys:CP ratio (6.01, 6.22, 6.45, 6.70, 6.97, or 7.26%; Table 8). There were 12 replications per treatment except for treatment 5 (SID Lys:CP of 6.97% with no DDGS) which had 11 replications. Dietary additions of feed-grade AA were adjusted to meet or exceed NRC (2012) AA requirement estimates in relation to Lys for Met and Cys, Thr, Trp, Val, Ile, and His. Both the low and high SID Lys:CP ratio diets, 6.01 and 7.26%, respectively, with and without DDGS, were manufactured at the Hubbard Feeds feed mill (Mankato, MN) in meal form and blended at the farm by an electronic feeding system (Dry Extract; Big Dutchman, Inc., Holland, MI) to make the intermediate dietary treatments. Diet samples were collected and analyzed for proximate analysis and complete AA profile at the University of Missouri Agricultural Experiment Station Chemical Laboratory (Table 9).

Pens of pigs were weighed and feed disappearance was measured at the beginning and end of the study (d 18) to calculate ADG, ADFI, and G:F. Daily feed additions were recorded by the electronic feeding system.

## Statistical Analysis

### Experiment 1

Growth, digestibility, and fecal DM data were analyzed as a randomized complete block design for one-way ANOVA using the lmer function from the lme4 package in R Studio (Version 4.3.1, R Core Team, Vienna, Austria) with pen serving as the experimental unit, dietary treatment as a fixed effect, and BW block as a random intercept. Linear and quadratic contrasts were tested within increasing levels of feed-grade AA without addition of added DAP or Gly. The effect of DAP and Gly were tested using pairwise comparisons to compare their responses to moderate and high levels of feed-grade AA individually. Blood urea N data were analyzed with pig serving as the observational unit, dietary treatment and gender as fixed effects, and block and microtiter plates as random intercepts. Pen was also included as a random effect to account for subsampling with multiple pigs measured from each pen. When treatment was a significant source of variation, differences were determined by pairwise comparison using the Tukey-Kramer multiplicity adjustment to control for Type I Error. For all experiments, results were considered significant with $P \leq 0.05$ and were considered marginally significant with $0.05 < P \leq 0.10$.

### Experiments 2 and 3

Data for Exp. 2 and 3 were analyzed as a randomized complete block design and a completely randomized design, respectively, for one-way ANOVA using the lmer function from the lme4 package in R Studio (Version 4.3.1, R Core Team, Vienna, Austria). For Exp. 2, feeder (two pens of pigs) was considered the experimental unit. Treatment was included in the model as a fixed effect and block was included in the model as a random intercept, which incorporated initial pen BW, sow farm origin, and date of entry into the nursery facility. For Exp. 3, pen was considered the experimental unit. Treatment was included in the model as a fixed effect and nursery entry date and room were included in the model as random effects to account for differences in genetic lines and fill date. Contrast statements were used to evaluate the interactive effect of SID Lys:CP ratio × SID Lys and SID Lys:CP ratio × DDGS for Exp. 2 and 3, respectively, as well as their main effects.

Dose–response curves were created using quadratic polynomial (QP) and broken-line linear (BLL) models in SAS OnDemand for Academics (SAS Institute, Inc., Cary, NC) using the NLMIXED and GLIMMIX procedures, respectively. Following model fit, the best-fitting model was selected using the Bayesian Information Criterion (BIC). A decrease in BIC greater than 2.0 among models for a particular response criterion was considered an improved fit. For Exp. 2, a broken line linear analysis was conducted for the effect of SID Lys:CP ratio on G:F in the low SID Lys diets (1.15%) to ensure SID Lys was below the requirement estimate. For Exp. 3, quadratic polynomial analyses were conducted for the effect of SID Lys:CP ratio on G:F within diets with and without 15% DDGS.

## Results

### Experiment 1

Overall (d 0 to 21), there was no evidence of differences in ADG due to dietary treatment (Table 10). A decrease in G:F was observed (linear, $P = 0.002$; quadratic, $P = 0.054$) with the greatest change occurring as the SID Lys:CP ratio increased from 6.5 to 7.0%. Incorporating either DAP or Gly to the high feed-grade AA diet led to an increase ($P \leq 0.003$) in G:F compared to the pigs fed the high feed-grade AA diet due to a reduction ($P = 0.007$) or numerical reduction ($P = 0.109$), respectively, in ADFI. No differences were observed between either DAP or Gly and the moderate feed-grade AA diet.

There was a decrease (linear, $P < 0.001$) in BUN as the SID Lys:CP ratio increased. Adding nitrogen to the high feed-grade AA diet in the form of DAP or Gly increased ($P = 0.038$) or tended to increase ($P = 0.091$) BUN, respectively, with no differences when compared to the moderate feed-grade AA diet.

There was a tendency ($P = 0.051$) for a quadratic effect in fecal DM with pigs fed the moderate feed-grade AA diet having the lowest fecal DM. Pigs fed the diet containing DAP had increased ($P = 0.005$) fecal DM compared with the pigs fed the high feed-grade AA diet. No differences were observed between either DAP or Gly and the moderate feed-grade AA diet.

There was a tendency for improvement ($P = 0.060$) in ATTD of DM for pigs fed the diet containing DAP compared to pigs fed the high feed-grade AA diet. Additionally, ATTD of CP decreased (linear, $P = 0.048$) with increasing feed-grade AA. Pigs fed diets containing either DAP or Gly had increased ($P \leq 0.026$) CP digestibility compared to pigs fed the high feed-grade AA diet. Pigs fed the diet containing DAP had increased ($P = 0.005$) CP digestibility compared to the pigs fed the moderate feed-grade AA diet.

## Experiment 2

Overall (d 0 to 14), a SID Lys:CP × SID Lys interaction was observed (linear, $P < 0.001$; Table 11) for G:F where increasing the SID Lys:CP ratio decreased G:F in both 1.15 and 1.30% SID Lys levels, with a greater magnitude of reduction observed in pigs fed diets formulated to 1.15% SID Lys (quadratic, $P = 0.016$) than in diets formulated to 1.30% SID Lys (linear, $P < 0.001$). An increase in ADG and d 14 BW was observed ($P \leq 0.002$) in pigs fed 1.30% SID Lys compared to those fed 1.15% SID Lys. Additionally, as SID Lys:CP ratio increased, ADFI increased ($P = 0.004$; Table 12). Dose response analysis indicated that maximum G:F was observed at 6.39% SID Lys:CP for the diets formulated to 1.15% SID Lys (Appendix Figure A).

## Experiment 3

Overall (d 0 to 18), a SID Lys:CP ratio × DDGS interaction was observed (linear, $P < 0.001$) for G:F (Table 13) driven by differences in SID Lys:CP in 0 and 15% DDGS. Pigs fed diets without DDGS had decreased G:F when fed diets with the lowest and highest SID Lys:CP ratios (quadratic, $P < 0.001$); whereas in pigs fed diets with DDGS, G:F decreased (linear, $P < 0.001$; quadratic, $P = 0.002$) as the SID Lys:CP ratio increased above 6.45%. For main effects (Table 14), ADG increased then decreased (quadratic, $P = 0.021$) as the SID Lys:CP ratio increased, with the greatest improvement in ADG observed in pigs fed diets with ratios of 6.45 to 6.97%. Additionally, ADFI increased (linear, $P = 0.018$) as SID Lys:CP increased. Pigs fed diets without DDGS tended to have increased ($P \leq 0.071$) ADG. Dose response analysis indicated that maximum G:F was observed at 6.69% SID Lys:CP for the corn-soy diets (0% DDGS; Appendix Figure B.1); whereas in 15% DDGS diets (Appendix Figure B.2), maximum G:F was observed at 6.47% SID Lys:CP.

# Discussion

As the swine industry continues to prioritize more sustainable and efficient production, optimizing N efficiency has become a key focus area. One of the major environmental concerns associated with pig production is N excretion, which is largely influenced by dietary CP. High CP diets significantly contribute to N waste, as excess protein that is not used for growth is excreted primarily in the form of urea and ammonia (Millet et al., 2018). A review of 33 swine metabolism studies suggests that N excretion decreases by approximately 8% for every 1 percentage unit reduction in CP, regardless of pig body weight (Kerr, 2003). Therefore, low protein, AA fortified diets are widely used in swine production. Lowering CP in swine diets has been shown to improve gut health by decreasing the incidence of diarrhea (Limbach et al., 2021), minimizing N excretion (Portejoie et al., 2004), and reducing feed costs by decreasing the amount of intact protein sources in diet. With the commercial availability of synthetic AA, it is possible to reduce dietary CP while still meeting EAA requirements. However, adequate N must still be provided to support the synthesis of NEAA. Therefore, formulating diets based on an optimal SID Lys:CP ratio could serve as a method to ensure sufficient N availability for NEAA synthesis, thereby maintaining pig performance.

Rocha et al. (2022) conducted a meta-analysis including data from 23 nursery experiments with pigs ranging from 5 to 36 kg and identified a SID Lys:CP ratio of 6.6% as the threshold above which ADG and G:F begin to decline. Although this meta-analysis combines studies involving pigs of varying body weights, the findings closely aligns with results from the present studies, in which maximum G:F was observed at a SID Lys:CP level of approximately 6.5%. Previously, Millet et al. (2018) evaluated the effects of CP and two dietary Lys levels that were intentionally formulated below the optimum level to ensure that Lys was the first limiting

AA. They reported an optimal SID Lys:CP ratio of 6.4% for pigs ranging from 8.3 kg to approximately 21.7 kg, above which growth performance declined. The findings of Millet et al. (2018) are consistent with the breakpoint analysis from Exp. 2, where optimal G:F was observed at a SID Lys:CP ratio of 6.39%. Overall, if the SID Lys:CP ratio is high and dietary CP is insufficient to supply adequate N, growth performance, particularly feed efficiency, can be compromised (Mansilla, 2013). A potential explanation for this reduction in growth performance is that the limited N provided from dietary CP prevents adequate synthesis of NEAA (Peng et al., 2016).

Non-protein nitrogen sources are rarely used in swine as monogastric animals cannot efficiently utilize NPN in the same manner as ruminants. However, NPN utilization as a strategy to limit N excretion has been of interest. Mansilla (2013) observed that G:F was reduced in pigs fed a diet deficient in NEAA compared to those supplemented with NPN in the form of urea and ammonium salts, showing that N from ammonia can be used as efficiently as intact protein as a source of N for NEAA synthesis. Urea has been the most studied NPN source in swine; however, ammonium salts have also been researched. Mansilla et al. (2017) demonstrated that a diet containing ammonium salts (diammonium phosphate; DAP at 1.2% and diammonium citrate; DAC at 1.7%) can effectively serve as a N source in a low protein diet. Mansilla et al. (2017) observed that increasing NPN inclusion improved G:F, with no differences observed between a high-NPN diet and the diet in which N was provided from intact protein sources. Amino acids can also be used as a N source for the synthesis of other NEAA by serving as a N donor during transamination reactions (Rezaei et al., 2013). Based on the findings of Exp. 1, growth performance can be maintained when a low protein diet, high in feed-grade AA is supplemented with a N source like DAP or Gly to support the synthesis of NEAA. These results agree with the work conducted by Mansilla et al. (2013; 2017).

Blood urea nitrogen serves as an indicator of protein metabolism and N balance, as urea is the main nitrogenous product of protein catabolism (Pedersen and Boisen, 2001). Consequently, BUN concentrations are directly affected by dietary CP level and AA in the diet. Elevated BUN levels typically suggest excessive dietary protein intake or an imbalance in AA composition, resulting in increased N excretion, while lower BUN concentrations can indicate improved N utilization or an inadequate supply of NEAA-N. In Exp. 1, BUN concentrations decreased as the SID Lys:CP ratio increased, indicating that there was excessive dietary CP intake and more N excretion at a lower SID Lys:CP level. Millet et al. (2018) similarly observed reductions in serum urea N concentrations with decreasing dietary CP. Additionally, in Exp. 1, pigs fed diets containing DAP and Gly had greater BUN concentrations compared to those fed the high feed-grade AA diet which also contained the lowest CP. This observation is logical, as DAP and Gly supply N, thus increasing the calculated CP of the diet. Interestingly, Whang and Easter (2000) reported that BUN concentrations are negatively correlated with G:F, indicating higher BUN was associated with poorer G:F. This disagrees with the results from Exp. 1 where G:F decreased as the SID Lys:CP ratio increased. A potential explanation for this inconsistency could be the imbalance of AA. At higher SID Lys:CP ratios, dietary N may become insufficient for the synthesis of NEAA, resulting in a deficiency that can impair growth performance. In such cases, pigs utilize the available N as efficiently as possible until NEAA become limiting, which is often reflected by a reduction in BUN concentrations.

Previous literature indicates that reducing CP in swine diets improves fecal consistency. Excess dietary CP can result in undigested protein fermentation in the hindgut, increasing microbial activity (Gao et al., 2020) and potentially leading to incidences of diarrhea (Marchetti et al., 2023). Htoo et al. (2007) observed that a low CP diet supplemented with AA reduced the proliferation of potentially harmful bacteria, which may explain the findings from Exp. 1, where the low CP diet tended to have the highest fecal DM. Similarly, other studies have reported a decrease in diarrhea in pigs fed lower CP diets (Heo et al., 2009; Limbach et al., 2021). Interestingly, when DAP was added to the high feed-grade AA diet, fecal DM was increased. Engelsmann et al. (2023) observed that higher feed intake was associated with a higher probability of diarrhea. This increase in fecal DM observed in pigs fed the diet supplemented with DAP may be attributed to the reduction in ADFI for this treatment relative to the high feed-grade AA diet. Another potential explanation may be the digestibility of DAP relative to intact protein. In the current study, pigs fed the DAP-supplemented diet had greater ATTD of CP than those fed diets without a supplemental N source. The N from DAP may be more bioavailable because it bypasses the need for proteolytic digestion required by intact proteins. As a result, less N reaches the hindgut for microbial fermentation, potentially reducing diarrhea incidence.

It is important to note that N digestibility in the current study, which was then converted to a CP basis, was determined using ATTD rather than SID. Apparent total tract digestibility for AA is generally considered less accurate as it does not account for endogenous losses (Stein et al., 2007). However, Mansilla (2013) suggested that fecal digestibility values are more accurate for estimating N bioavailability compared to SID values. This is because N absorbed by the lower gut, primarily in the form of ammonia due to microbial fermentation, can be efficiently absorbed by the mucosa in the lower gut for body protein deposition (Mansilla, 2013). Mansilla (2013) observed that the ATTD of DM was greater for pigs with a high urea-N infusion than those infused with saline. Although pigs were not infused in the present study, the ATTD of DM tended to be higher in pigs fed DAP compared to those on the high feed-grade AA diet without added N. Mansilla (2013) had noted enhanced N utilization for pigs infused with urea, which likely improved overall nutrient absorption and utilization, including DM. Additionally in Exp. 1, a linear reduction in ATTD of CP was observed with increasing SID Lys:CP or lowering CP in the diets. Petersen et al. (2014) observed that corn has decreased SID CP (80.1%) compared to SBM (85.9%). Therefore, a potential explanation for this response could be the change in diet composition. As the SID Lys:CP ratio increases, corn inclusion, which is less digestible compared to SBM, increases, while SBM decreases. Furthermore, when DAP and Gly were supplemented to the high feed-grade AA diet without added N, there was an increased in the ATTD of CP, likely because these N sources are more bioavailable than N provided by an intact protein source. Intact proteins must undergo proteolytic digestion by enzymes to be absorbed (Rivest et al., 2000). Because N from NPN or amino N are already in a simplified form, there may be quicker absorption in the gastrointestinal tract.

The NRC (2012) suggests a SID Lys requirement of 1.23% for pigs weighing between 11 and 25 kg. In Exp. 1, all diets were formulated to a SID Lys level of 1.25%, which is similar to the NRC (2012) estimate for pigs in this weight range. However, the pigs would be below the requirement estimate of 11.1 g/d SID Lys based on feed intake, as the treatment with the highest ADFI (7.0% SID Lys:CP) would have only consumed 10.8 g/d SID Lys. Many studies have evaluated the optimal SID Lys level for pigs in this weight range. Kendall et al. (2008) conducted five experiments and estimated a requirement of 1.30% SID Lys for pigs weighing 11 to 27 kg. However, there are few studies evaluating the effect of SID Lys:CP in diets that are adequate or limiting in SID Lys. Therefore, Exp. 2 evaluated the effect of SID Lys both below (1.15%) and above (1.30%) the NRC requirement estimate to better determine the optimal SID

Lys:CP ratio. Millet et al. (2018) evaluated the effects of varying dietary CP concentrations and two SID Lys levels (1.0 and 1.1%) in pigs weaned at 8.3 kg. They observed that ADG increased as the CP level increased, possibly due to the availability of N in the diets. Additionally, Millet et al. (2018) observed an interaction where at low CP levels, the performance differences between the two SID Lys levels were minimal, possibly because N was first limiting before Lys. At higher CP levels, pigs fed diets with increased SID Lys exhibited increased G:F. In Exp. 2, increasing SID Lys improved overall pig performance. Additionally, the observed SID Lys:CP × SID Lys interaction suggests that pigs fed 1.15% SID Lys were more responsive to increasing SID Lys:CP ratio than those fed 1.30% SID Lys, likely because they were further below their SID Lys requirement. This data suggests that when SID Lys is provided at an adequate level, the SID Lys:CP ratio becomes less critical compared to when both SID Lys and N are limiting.

Previous data suggests that high SBM levels in diets for 10 to 25 kg pigs can impair growth performance (Faccin et al., 2024). Thus, decreased performance at 6.01% SID Lys:CP in the corn-soy diet in Exp. 3 may be attributed to the high SBM content (37.8%). Therefore, the optimal SID Lys:CP ratio may be confounded with the SBM concentration within the diet. In Exp. 3, the SID Lys:CP × DDGS interaction for G:F indicates that the response to SID Lys:CP differed between the two DDGS levels, which may be driven by the high SBM content of the corn-SBM diet without DDGS at 6.01% SID Lys:CP. For diets containing 15% DDGS, G:F peaked at 6.47% SID Lys:CP, aligning with data from Exp. 1 and 2 and previous literature evaluating SID Lys:CP ratios (Millet et al., 2018; Rocha et al., 2022). In the corn-SBM diets without DDGS, maximum G:F was observed at a calculated SID Lys:CP level of 6.69%. It is important to note that these ratios are calculated values based on the analyzed composition of ingredients (SBM, corn, and DDGS) before the study. A potential explanation for the slightly higher estimate for SID Lys:CP could be that the calculated CP content of diets from analyzed ingredients was slightly lower than CP of analyzed diets. Thus, the estimated SID Lys:CP ratios shift slightly. The corn-SBM diet formulated to 6.70% SID Lys:CP would have an approximate calculated ratio of 6.60% while the 6.45% SID Lys:CP diet with DDGS has a calculated ratio of approximately 6.52%. Nevertheless, main effects indicate that maximal G:F was observed at 6.45% SID Lys:CP, which is consistent with the previous literature (Millet et al., 2018; Rocha et al., 2022).

Instead of using total CP, formulating diets based on SID Lys:SID CP may serve as a strategy to ensure sufficient N availability for NEAA synthesis. The NRC (2012) estimates a SID N requirement of 2.56% for 11- to 25-kg pigs, which corresponds to a calculated ratio of 7.69% SID Lys:SID CP. Expressing CP on a SID basis would improve precision by estimating digestible N available to the pig; however, this approach is not commonly used in industry practice. In Exp. 1, the moderate feed-grade AA diet had a SID Lys:SID CP ratio of 7.68%, beyond which G:F decreased. A similar trend was observed in Exp. 2, where growth performance decreased when the SID Lys:SID CP ratio exceeded 7.60%. In Exp. 3, maximum G:F was observed at a SID Lys:SID CP ratio of 7.88% in the 0% DDGS diets and 7.59% in the 15% DDGS diets. Either method supports the concept that a high SID Lys:CP or SID Lys:SID CP ratio may result in insufficient N for NEAA synthesis and impair growth performance.

In summary, these data suggest that a SID Lys:CP ratio of approximately 6.5% is optimal for pigs weighing 11- to 25-kg, aligning closely with the calculated NRC (2012) estimate. On an SID CP basis this equates to a SID Lys:SID CP ratio of approximately 7.7%. When the ratio exceeds this value, growth performance, particularly feed efficiency, is compromised likely because N from CP becomes limiting for the synthesis of NEAA. Feed efficiency can be recovered by incorporating a N source, such as NPN like DAP or amino N sources like Gly.

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**Table 1.1.** Analyzed composition of soybean meal (SBM) and corn for Exp. 1 (as-fed basis)[1]

| Nutrient, % | SBM | Corn |
|---|---|---|
| CP | 46.09 | 7.23 |
| Dry matter | 86.97 | 84.77 |
| Crude fat | 0.58 | 3.60 |
| Crude fiber | 3.86 | 1.77 |
| Ash | 5.77 | 1.10 |
| Amino acids | | |
| Arg | 3.14 | 0.30 |
| Cys | 0.57 | 0.15 |
| His | 1.15 | 0.19 |
| Ile | 1.97 | 0.23 |
| Leu | 3.32 | 0.88 |
| Lys | 2.75 | 0.23 |
| Met | 0.57 | 0.14 |
| Phe | 2.17 | 0.27 |
| Thr | 1.77 | 0.25 |
| Trp | 0.63 | 0.05 |
| Val | 2.10 | 0.32 |

[1] Samples were submitted to Market 1, AgState (Cherokee, IA) for proximate analysis and AA profile using NIR and a ring test was performed to validate the values against wet chemistry.

**Table 1.2.** Diet composition, Exp. 1 (as-fed basis)[1]

| Feed-grade AA: | Low | Moderate | High | High | High |
|---|---|---|---|---|---|
| Added N: | None | None | None | DAP[2] | Glycine |
| SID Lys:CP: | 6.0:1 | 6.5:1 | 7.0:1 | 6.5:1 | 6.5:1 |
| Ingredient, % | | | | | |
| Corn | 62.12 | 66.78 | 70.10 | 69.52 | 68.97 |
| Soybean meal | 33.75 | 28.55 | 24.74 | 24.74 | 24.74 |
| Calcium carbonate | 0.68 | 0.70 | 0.70 | 1.20 | 0.70 |
| Monocalcium P (21% P) | 0.95 | 1.00 | 1.05 | --- | 1.05 |
| Salt | 0.60 | 0.60 | 0.61 | 0.61 | 0.61 |
| L-Lys-HCl | 0.40 | 0.55 | 0.66 | 0.66 | 0.66 |
| DL-Met | 0.25 | 0.29 | 0.32 | 0.32 | 0.32 |
| L-Trp | 0.04 | 0.06 | 0.09 | 0.09 | 0.09 |
| L-Val | 0.13 | 0.21 | 0.28 | 0.28 | 0.28 |
| L-Ile | --- | 0.06 | 0.13 | 0.13 | 0.13 |
| Thr[3] | 0.27 | 0.35 | 0.42 | 0.42 | 0.42 |
| L-His-HCl | --- | 0.03 | 0.08 | 0.08 | 0.08 |
| Vitamin premix with phytase[4,5] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Trace mineral premix[6] | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Glycine | --- | --- | --- | --- | 1.14 |
| Diammonium phosphate (18% N) | --- | --- | --- | 1.14 | --- |
| Titanium dioxide | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Copper chloride[7] | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| Total | 100 | 100 | 100 | 100 | 100 |
| Calculated analysis | | | | | |
| SID AA | | | | | |
| Lys, % | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 |
| Ile:Lys | 57 | 55 | 55 | 55 | 55 |
| Leu:Lys | 117 | 108 | 101 | 100 | 100 |
| Met:Lys | 39 | 40 | 42 | 42 | 42 |
| Met and Cys:Lys | 58 | 58 | 58 | 58 | 58 |
| Thr:Lys | 67 | 67 | 67 | 67 | 67 |
| Trp:Lys | 20.3 | 20.0 | 20.3 | 20.3 | 20.3 |
| Val:Lys | 72 | 72 | 72 | 72 | 72 |
| His:Lys | 36 | 34 | 34 | 34 | 34 |
| Arg:Lys | 93 | 81 | 73 | 73 | 73 |
| Phe and Tyr:Lys | 111 | 99 | 90 | 90 | 90 |
| Total Lys, % | 1.39 | 1.38 | 1.37 | 1.37 | 1.37 |
| SID EAA:NEAA | 0.84 | 0.89 | 0.94 | 0.94 | 0.83 |
| NE, kcal/kg | 2,509 | 2,525 | 2,537 | 2,521 | 2,530 |
| SID Lys:NE, g/Mcal | 4.98 | 4.95 | 4.93 | 4.95 | 4.93 |
| CP, % | 20.9 | 19.2 | 17.9 | 19.2 | 19.2 |
| Ca, % | 0.63 | 0.63 | 0.63 | 0.63 | 0.63 |
| STTD P, % | 0.47 | 0.47 | 0.47 | 0.47 | 0.47 |
| Ca:P | 1.03 | 1.06 | 1.07 | 1.08 | 1.07 |

[1] Diets were fed from 10.3 to 22.0 kg.

[2] Diammonium phosphate.

[3] Thr Pro; CJ America-Bio, Downers Grove, IL.

[4] Provided per kg of diet: 4,134 IU vitamin A; 1,653 IU vitamin D; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin B12; 50 mg niacin; 28 mg pantothenic acid; 8 mg riboflavin.

[5] Empirical phytase (ADM, Chicago, IL) included at 1,250 FTU/kg provided an estimated release of 0.13% STTD P.

[6] Provided per kg of diet: 0.02 g copper; 0.30 mg iodine; 0.11 g iron; 0.03 g manganese; 0.30 mg selenium; 0.11 g zinc.

[7] Tribasic copper chloride, 58% Cu; SAM Nutrition, Bloomington, MN.

**Table 1.3.** Analyzed composition of Exp. 1 diets (as-fed basis)[1]

| Feed-grade AA: | Low | Moderate | High | High | High |
|---|---|---|---|---|---|
| Added N: | None | None | None | DAP[2] | Glycine |
| SID Lys:CP: | 6.0:1 | 6.5:1 | 7.0:1 | 6.5:1 | 6.5:1 |
| Nutrient, % | | | | | |
| CP | 19.24 | 19.04 | 17.21 | 19.16 | 18.53 |
| Moisture | 12.73 | 13.20 | 13.04 | 13.02 | 12.89 |
| Crude fat | 2.28 | 1.97 | 2.22 | 2.15 | 2.38 |
| Crude fiber | 2.03 | 1.90 | 2.02 | 2.05 | 2.03 |
| Ash | 5.55 | 5.39 | 5.13 | 5.06 | 4.88 |
| Essential AA | | | | | |
| Arg | 1.20 | 1.17 | 1.02 | 0.95 | 0.97 |
| His | 0.49 | 0.50 | 0.49 | 0.45 | 0.48 |
| Ile | 0.82 | 0.86 | 0.82 | 0.78 | 0.77 |
| Leu | 1.59 | 1.57 | 1.45 | 1.39 | 1.42 |
| Lys | 1.37 | 1.46 | 1.39 | 1.33 | 1.33 |
| Met | 0.47 | 0.56 | 0.55 | 0.49 | 0.53 |
| Phe | 0.92 | 0.91 | 0.82 | 0.77 | 0.78 |
| Thr | 0.89 | 0.93 | 0.93 | 0.84 | 0.87 |
| Trp | 0.27 | 0.27 | 0.27 | 0.28 | 0.27 |
| Val | 1.02 | 1.07 | 1.02 | 0.98 | 0.99 |
| Non-essential AA | | | | | |
| Ala | 0.93 | 0.92 | 0.85 | 0.82 | 0.85 |
| Asp | 1.92 | 1.88 | 1.66 | 1.56 | 1.56 |
| Cys | 0.29 | 0.30 | 0.27 | 0.24 | 0.26 |
| Glu | 3.39 | 3.34 | 3.02 | 2.83 | 2.86 |
| Gly | 0.77 | 0.75 | 0.68 | 0.63 | 1.64 |
| Pro | 1.09 | 1.08 | 1.01 | 0.96 | 1.00 |
| Ser | 0.81 | 0.78 | 0.70 | 0.67 | 0.70 |
| Tyr | 0.64 | 0.63 | 0.55 | 0.54 | 0.55 |

[1] Samples were analyzed for proximate analysis and complete AA profile (University of Missouri Agricultural Experiment Station Chemical Laboratory).

[2] Diammonium phosphate.

**Table 1.4.** Analyzed composition of soybean meal (SBM) and corn for Exp. 2 (as-fed basis)[1]

| Nutrient, % | SBM | Corn |
|---|---|---|
| CP | 45.10 | 7.02 |
| Dry matter | 88.47 | 86.52 |
| Crude fat | 1.42 | 2.42 |
| Crude fiber | 3.44 | 1.68 |
| Ash | 6.04 | 1.07 |
| Essential AA | | |
| Arg | 3.32 | 0.30 |
| His | 1.23 | 0.20 |
| Ile | 2.21 | 0.25 |
| Leu | 3.62 | 0.85 |
| Lys | 3.02 | 0.23 |
| Met | 0.63 | 0.15 |
| Phe | 2.45 | 0.35 |
| Thr | 1.82 | 0.25 |
| Trp | 0.64 | 0.05 |
| Val | 2.27 | 0.33 |
| Non-essential AA | | |
| Ala | 2.01 | 0.51 |
| Asp | 5.26 | 0.46 |
| Cys | 0.69 | 0.17 |
| Glu | 8.60 | 1.32 |
| Gly | 1.94 | 0.26 |
| Pro | 2.38 | 0.64 |
| Ser | 2.03 | 0.32 |
| Tyr | 1.73 | 0.20 |

[1] Samples were analyzed for proximate analysis and complete AA profile (University of Missouri Agricultural Experiment Station Chemical Laboratory).

**Table 1.5.** Diet composition, Exp. 2 (as-fed basis)[1]

| SID Lys, %: | 1.15 | | 1.30 | |
|---|---|---|---|---|
| SID Lys:CP: | 6.00 | 7.00 | 6.00 | 7.00 |
| Ingredient, % | | | | |
| Corn | 64.73 | 72.40 | 58.32 | 66.86 |
| Soybean meal | 31.44 | 22.81 | 37.94 | 28.32 |
| Calcium carbonate | 0.93 | 0.90 | 0.94 | 0.91 |
| Monocalcium P (21.5% P) | 0.95 | 1.05 | 0.85 | 0.97 |
| Salt | 0.30 | 0.30 | 0.30 | 0.30 |
| Liquid Lys (55% Lys) | 0.35 | 0.75 | 0.33 | 0.77 |
| DL-Met | 0.14 | 0.22 | 0.17 | 0.26 |
| L-Trp | 0.01 | 0.06 | 0.01 | 0.06 |
| L-Val | 0.01 | 0.16 | --- | 0.18 |
| L-Ile | --- | 0.05 | --- | 0.04 |
| L-Thr | 0.18 | 0.32 | 0.19 | 0.35 |
| L-His | --- | 0.03 | --- | 0.03 |
| Sodium metabisulfate | 0.50 | 0.50 | 0.50 | 0.50 |
| Zinc oxide | 0.10 | 0.10 | 0.10 | 0.10 |
| Copper sulfate | 0.07 | 0.07 | 0.07 | 0.07 |
| Trace mineral premix[2] | 0.10 | 0.10 | 0.10 | 0.10 |
| Vitamin premix[3] | 0.10 | 0.10 | 0.10 | 0.10 |
| Phytase[4] | 0.10 | 0.10 | 0.10 | 0.10 |
| Total | 100 | 100 | 100 | 100 |
| Calculated analysis | | | | |
| SID AA | | | | |
| Lys, % | 1.15 | 1.15 | 1.30 | 1.30 |
| Ile:Lys | 65 | 56 | 67 | 56 |
| Leu:Lys | 129 | 110 | 126 | 107 |
| Met:Lys | 35 | 38 | 35 | 38 |
| Met and Cys:Lys | 58 | 58 | 58 | 58 |
| Thr:Lys | 65 | 65 | 65 | 65 |
| Trp:Lys | 19.0 | 19.2 | 19.2 | 19.3 |
| Val:Lys | 70 | 70 | 70 | 70 |
| His:Lys | 39.6 | 34.1 | 39.8 | 34.2 |
| Total Lys, % | 1.29 | 1.27 | 1.46 | 1.43 |
| SID EAA:NEAA | 0.88 | 0.98 | 0.88 | 0.98 |
| NE, kcal/kg | 2,505 | 2,530 | 2,491 | 2,518 |
| SID Lys:NE, g/Mcal | 4.59 | 4.54 | 5.22 | 5.16 |
| CP, % | 19.2 | 16.4 | 21.7 | 18.6 |
| Ca, % | 0.66 | 0.64 | 0.66 | 0.65 |
| STTD P, % | 0.47 | 0.47 | 0.47 | 0.47 |
| Ca:P | 1.10 | 1.10 | 1.10 | 1.10 |

[1] Diets were fed from 13.0 to 26.4 kg.

[2] Provided per kg of diet: 0.02 g copper; 0.40 mg iodine; 0.11 g iron; 0.03 g manganese; 0.30 mg selenium; 0.11 g zinc.

[3] Provided per kg of diet: 3,307 IU vitamin A; 1,323 IU vitamin D; 35 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin B12; 44 mg niacin; 22 mg pantothenic acid; 6 mg riboflavin; 6 mg thiamin.

[4] Quantum Blue 2G (AB Vista, Marlborough, Wiltshire, UK) included at 2,000 FTU/kg provided an estimated release of 0.14% STTD P.

**Table 1.6.** Analyzed composition of Exp. 2 diets (as-fed basis)[1]

| SID Lys, %: | 1.15 | | | | | 1.30 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SID Lys:CP: | 6.00 | 6.22 | 6.46 | 6.72 | 7.00 | 6.00 | 6.22 | 6.46 | 6.72 | 7.00 |
| Nutrient, % | | | | | | | | | | |
| CP | 19.41 | 19.24 | 17.60 | 17.31 | 16.37 | 22.37 | 20.45 | 20.11 | 19.09 | 18.97 |
| Dry matter | 87.68 | 87.95 | 87.15 | 87.15 | 87.10 | 87.71 | 87.63 | 87.55 | 87.58 | 87.37 |
| Crude fat | 1.24 | 1.39 | 1.55 | 1.54 | 1.64 | 1.16 | 1.13 | 1.03 | 1.36 | 1.38 |
| Crude fiber | 2.18 | 2.07 | 2.06 | 1.87 | 1.82 | 2.04 | 1.98 | 1.90 | 1.93 | 2.02 |
| Ash | 4.78 | 4.81 | 4.68 | 4.76 | 4.53 | 5.15 | 4.97 | 5.14 | 4.64 | 4.60 |
| Essential AA | | | | | | | | | | |
| Arg | 1.22 | 1.18 | 1.07 | 1.03 | 1.01 | 1.47 | 1.28 | 1.27 | 1.18 | 1.16 |
| His | 0.50 | 0.50 | 0.46 | 0.45 | 0.45 | 0.59 | 0.53 | 0.53 | 0.51 | 0.52 |
| Ile | 0.85 | 0.83 | 0.77 | 0.77 | 0.75 | 1.01 | 0.90 | 0.90 | 0.86 | 0.85 |
| Leu | 1.60 | 1.57 | 1.47 | 1.43 | 1.41 | 1.86 | 1.67 | 1.66 | 1.58 | 1.57 |
| Lys | 1.27 | 1.29 | 1.24 | 1.28 | 1.27 | 1.48 | 1.38 | 1.43 | 1.38 | 1.41 |
| Met | 0.42 | 0.43 | 0.42 | 0.48 | 0.50 | 0.49 | 0.49 | 0.54 | 0.51 | 0.55 |
| Phe | 0.94 | 0.91 | 0.84 | 0.82 | 0.80 | 1.12 | 0.99 | 0.98 | 0.92 | 0.90 |
| Thr | 0.85 | 0.85 | 0.84 | 0.85 | 0.86 | 0.98 | 0.94 | 0.98 | 0.91 | 0.93 |
| Trp | 0.19 | 0.21 | 0.19 | 0.20 | 0.20 | 0.24 | 0.21 | 0.23 | 0.23 | 0.21 |
| Val | 0.96 | 0.97 | 0.94 | 0.94 | 0.94 | 1.11 | 1.03 | 1.06 | 1.06 | 1.09 |
| Non-essential AA | | | | | | | | | | |
| Ala | 0.93 | 0.92 | 0.86 | 0.83 | 0.83 | 1.07 | 0.97 | 0.95 | 0.92 | 0.91 |
| Asp | 1.90 | 1.84 | 1.68 | 1.62 | 1.57 | 2.31 | 2.00 | 1.99 | 1.84 | 1.82 |
| Cys | 0.31 | 0.30 | 0.28 | 0.27 | 0.27 | 0.35 | 0.31 | 0.31 | 0.30 | 0.30 |
| Glu | 3.45 | 3.37 | 3.12 | 3.02 | 2.94 | 4.11 | 3.64 | 3.60 | 3.39 | 3.34 |
| Gly | 0.77 | 0.75 | 0.70 | 0.67 | 0.66 | 0.91 | 0.81 | 0.80 | 0.75 | 0.74 |
| Pro | 1.08 | 1.05 | 1.00 | 0.98 | 0.97 | 1.21 | 1.11 | 1.10 | 1.07 | 1.06 |
| Ser | 0.77 | 0.75 | 0.70 | 0.67 | 0.64 | 0.89 | 0.80 | 0.79 | 0.75 | 0.72 |
| Tyr | 0.64 | 0.61 | 0.58 | 0.56 | 0.55 | 0.74 | 0.67 | 0.66 | 0.62 | 0.62 |

[1] Samples were analyzed for proximate analysis and complete AA profile (University of Missouri Agricultural Experiment Station Chemical Laboratory).

**Table 1.7.** Analyzed composition of corn, soybean meal (SBM), and dried distillers grains with solubles (DDGS) for Exp. 3 (as-fed basis)[1]

| Nutrient, % | Corn | SBM | DDGS |
|---|---|---|---|
| CP | 6.76 | 45.64 | 28.50 |
| Dry matter | 85.66 | 89.76 | 88.89 |
| Crude fat | 2.62 | 1.98 | 8.30 |
| Crude fiber | 1.61 | 4.08 | 7.53 |
| Ash | 1.10 | 6.51 | 4.96 |
| Essential AA | | | |
| Arg | 0.34 | 3.30 | 1.28 |
| His | 0.20 | 1.22 | 0.81 |
| Ile | 0.25 | 2.27 | 1.07 |
| Leu | 0.77 | 3.59 | 3.05 |
| Lys | 0.27 | 3.02 | 0.96 |
| Met | 0.15 | 0.67 | 0.49 |
| Phe | 0.33 | 2.43 | 1.31 |
| Thr | 0.25 | 1.80 | 1.07 |
| Trp | 0.05 | 0.63 | 0.21 |
| Val | 0.34 | 2.36 | 1.42 |
| Non-essential AA | | | |
| Ala | 0.50 | 2.04 | 1.80 |
| Asp | 0.49 | 5.33 | 1.77 |
| Cys | 0.16 | 0.71 | 0.54 |
| Glu | 1.25 | 8.65 | 3.73 |
| Gly | 0.29 | 1.99 | 1.09 |
| Pro | 0.62 | 2.39 | 2.04 |
| Ser | 0.30 | 1.98 | 1.18 |
| Tyr | 0.23 | 1.76 | 1.03 |

[1] Samples were analyzed for proximate analysis and complete AA profile (University of Missouri Agricultural Experiment Station Chemical Laboratory).

**Table 1.8.** Diet composition, Exp. 3 (as-fed basis)[1]

| | No DDGS | | 15% DDGS | |
|---|---|---|---|---|
| SID Lys:CP: | 6.01 | 7.26 | 6.01 | 7.26 |
| Ingredient, % | | | | |
| Corn | 59.03 | 69.18 | 52.99 | 63.18 |
| Soybean meal | 37.79 | 26.48 | 28.70 | 17.24 |
| Corn DDGS (8.3% oil) | --- | --- | 15.00 | 15.00 |
| Calcium carbonate | 0.95 | 0.92 | 1.04 | 1.00 |
| Monocalcium P (21.5% P) | 0.85 | 1.05 | 0.65 | 0.83 |
| Salt | 0.60 | 0.61 | 0.54 | 0.55 |
| L-Lys | 0.21 | 0.57 | 0.43 | 0.79 |
| DL-Met | 0.15 | 0.27 | 0.16 | 0.27 |
| L-Thr | 0.15 | 0.31 | 0.19 | 0.35 |
| L-Trp | 0.01 | 0.07 | 0.04 | 0.10 |
| L-Val | --- | 0.18 | 0.01 | 0.23 |
| L-Ile | --- | 0.06 | --- | 0.14 |
| L-His | --- | 0.05 | --- | 0.08 |
| Trace mineral premix[2] | 0.08 | 0.08 | 0.08 | 0.08 |
| Vitamin premix[3] | 0.05 | 0.05 | 0.05 | 0.05 |
| Copper sulfate | 0.07 | 0.07 | 0.07 | 0.07 |
| Selenium | 0.05 | 0.05 | 0.05 | 0.05 |
| Phytase[4] | 0.02 | 0.02 | 0.02 | 0.02 |
| Total | 100 | 100 | 100 | 100 |
| Calculated analysis | | | | |
| SID AA, % | | | | |
| Lys, % | 1.30 | 1.30 | 1.30 | 1.30 |
| Ile:Lys | 68 | 56 | 62 | 56 |
| Leu:Lys | 122 | 100 | 127 | 104 |
| Met:Lys | 35 | 39 | 35 | 39 |
| Met and Cys:Lys | 58 | 58 | 58 | 58 |
| Thr:Lys | 65 | 65 | 65 | 65 |
| Trp:Lys | 19 | 19 | 19 | 19 |
| Val:Lys | 72 | 70 | 70 | 70 |
| His:Lys | 39 | 34 | 38 | 34 |
| Total Lys, % | 1.47 | 1.44 | 1.49 | 1.46 |
| SID EAA:NEAA | 0.88 | 0.99 | 0.92 | 1.07 |
| NE, kcal/kg | 2,504 | 2,537 | 2,489 | 2,526 |
| SID Lys:NE, g/Mcal | 5.19 | 5.12 | 5.22 | 5.15 |
| CP, % | 21.6 | 17.9 | 21.6 | 17.9 |
| Ca, % | 0.67 | 0.65 | 0.65 | 0.63 |
| STTD P, % | 0.45 | 0.46 | 0.45 | 0.45 |
| Ca:P | 1.10 | 1.10 | 1.10 | 1.10 |

[1] Diets were fed from 11.0 to 20.4 kg.

[2] Provided per kg of diet: 0.01 g copper; 0.53 mg iodine; 0.11 g iron; 0.03 g manganese; 0.13 g zinc.

[3] Provided per kg of diet: 12 IU vitamin A; 2.2 IU vitamin D3; 66 IU vitamin E; 7 mg vitamin K; 0.06 mg vitamin B12; 50 mg niacin; 45 mg pantothenic acid; 9 mg riboflavin; 1 mg thiamin.

[4] Quantum Blue 5G (AB Vista, Marlborough, Wiltshire, UK) included at 751 FTU/kg provided an estimated release of 0.12% STTD P.

**Table 1.9.** Analyzed composition of Exp. 3 diets (as-fed basis)[1]

| | No DDGS | | | | | | DDGS | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SID Lys:CP: | 6.01 | 6.22 | 6.45 | 6.70 | 6.97 | 7.26 | 6.01 | 6.22 | 6.45 | 6.70 | 6.97 | 7.26 |
| Nutrient, % | | | | | | | | | | | | |
| CP | 22.70 | 20.94 | 19.95 | 19.69 | 19.44 | 18.55 | 21.81 | 21.08 | 19.94 | 19.86 | 18.73 | 18.73 |
| Dry matter | 87.73 | 87.84 | 87.83 | 87.66 | 87.57 | 87.88 | 88.63 | 88.48 | 88.63 | 88.45 | 88.25 | 88.36 |
| Crude fat | 1.90 | 2.04 | 2.17 | 1.75 | 1.53 | 1.69 | 2.52 | 2.70 | 2.88 | 2.69 | 3.02 | 3.01 |
| Crude fiber | 2.36 | 2.40 | 2.40 | 1.89 | 1.70 | 1.72 | 2.77 | 2.74 | 2.69 | 3.07 | 2.80 | 2.93 |
| Ash | 5.33 | 4.93 | 5.02 | 5.81 | 5.03 | 4.50 | 5.68 | 5.35 | 5.30 | 5.43 | 5.22 | 5.32 |
| Essential AA | | | | | | | | | | | | |
| Arg | 1.48 | 1.36 | 1.30 | 1.23 | 1.21 | 1.12 | 1.31 | 1.26 | 1.21 | 1.15 | 1.05 | 1.05 |
| His | 0.63 | 0.59 | 0.59 | 0.57 | 0.56 | 0.53 | 0.61 | 0.60 | 0.60 | 0.59 | 0.57 | 0.60 |
| Ile | 1.01 | 0.94 | 0.91 | 0.90 | 0.87 | 0.81 | 0.95 | 0.91 | 0.92 | 0.88 | 0.85 | 0.86 |
| Leu | 1.84 | 1.75 | 1.68 | 1.63 | 1.59 | 1.53 | 1.96 | 1.90 | 1.86 | 1.78 | 1.71 | 1.73 |
| Lys | 1.50 | 1.44 | 1.44 | 1.51 | 1.52 | 1.42 | 1.53 | 1.49 | 1.51 | 1.46 | 1.46 | 1.51 |
| Met | 0.41 | 0.45 | 0.39 | 0.44 | 0.46 | 0.42 | 0.49 | 0.47 | 0.46 | 0.44 | 0.43 | 0.46 |
| Phe | 1.14 | 1.06 | 1.01 | 0.98 | 0.96 | 0.89 | 1.10 | 1.06 | 1.02 | 0.97 | 0.91 | 0.91 |
| Thr | 1.00 | 0.92 | 0.94 | 0.96 | 0.97 | 0.93 | 0.99 | 0.99 | 0.97 | 0.94 | 0.90 | 0.95 |
| Trp | 0.26 | 0.25 | 0.27 | 0.27 | 0.25 | 0.25 | 0.25 | 0.26 | 0.27 | 0.26 | 0.27 | 0.26 |
| Val | 1.10 | 1.08 | 1.04 | 1.05 | 1.06 | 1.01 | 1.07 | 1.08 | 1.09 | 1.05 | 1.05 | 1.06 |
| Non-essential AA | | | | | | | | | | | | |
| Ala | 1.07 | 1.02 | 0.98 | 0.94 | 0.92 | 0.89 | 1.15 | 1.13 | 1.10 | 1.05 | 1.01 | 1.03 |
| Asp | 2.32 | 2.12 | 2.03 | 1.94 | 1.91 | 1.74 | 2.05 | 1.96 | 1.87 | 1.75 | 1.63 | 1.62 |
| Cys | 0.37 | 0.35 | 0.33 | 0.32 | 0.31 | 0.30 | 0.38 | 0.38 | 0.36 | 0.35 | 0.33 | 0.35 |
| Glu | 4.10 | 3.82 | 3.66 | 3.53 | 3.46 | 3.24 | 3.88 | 3.75 | 3.66 | 3.43 | 3.25 | 3.26 |
| Gly | 0.93 | 0.86 | 0.82 | 0.78 | 0.77 | 0.73 | 0.88 | 0.86 | 0.83 | 0.78 | 0.73 | 0.74 |
| Pro | 1.24 | 1.19 | 1.14 | 1.10 | 1.08 | 1.05 | 1.35 | 1.32 | 1.28 | 1.23 | 1.19 | 1.21 |
| Ser | 0.97 | 0.90 | 0.87 | 0.82 | 0.80 | 0.77 | 0.93 | 0.92 | 0.86 | 0.80 | 0.78 | 0.79 |
| Tyr | 0.79 | 0.74 | 0.72 | 0.69 | 0.67 | 0.63 | 0.78 | 0.76 | 0.73 | 0.70 | 0.66 | 0.67 |

[1] Samples were analyzed for proximate analysis and complete AA profile (University of Missouri Agricultural Experiment Station Chemical Laboratory).

**Table 1.10.** Effect of feed-grade amino acid (AA) inclusion with and without an added nitrogen (N) source on growth performance, serum blood urea nitrogen (BUN), fecal dry matter (DM), and digestibility of DM and crude protein (CP; Exp. 1)[1]

| Feed-grade AA: | Low | Moderate | High | High | High | | *P* = | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Added N: | None | None | None | DAP[2] | Glycine | | SID Lys:CP[3] | | | |
| SID Lys:CP: | 6.0:1 | 6.5:1 | 7.0:1 | 6.5:1 | 6.5:1 | SEM | Linear | Quadratic | DAP[4] | Glycine[5] |
| BW, kg | | | | | | | | | | |
| d 0 | 10.3 | 10.3 | 10.3 | 10.4 | 10.4 | 0.19 | 0.715 | 0.649 | 0.354 | 0.297 |
| d 21 | 22.2 | 22.1 | 22.0 | 21.8 | 22.1 | 0.32 | 0.344 | 0.965 | 0.377 | 0.518 |
| Overall (d 0 to 21) | | | | | | | | | | |
| ADG, g | 562 | 560 | 557 | 545 | 560 | 8.8 | 0.616 | 0.989 | 0.280 | 0.736 |
| ADFI, g | 841 | 838 | 864 | 818 | 838 | 14.3 | 0.163 | 0.291 | 0.007 | 0.109 |
| G:F, g/kg | 668 | 669 | 645 | 667 | 669 | 4.9 | 0.002 | 0.054 | 0.003 | 0.001 |
| Blood urea N, mg/dL | 8.17 | 7.12 | 6.27 | 6.88 | 6.76 | 0.224 | < 0.001 | 0.680 | 0.038 | 0.091 |
| Fecal DM, % | 20.49 | 19.61 | 21.99 | 24.81 | 22.87 | 0.660 | 0.118 | 0.051 | 0.005 | 0.351 |
| ATTD DM, % | 83.88 | 84.08 | 83.82 | 85.55 | 84.70 | 0.630 | 0.951 | 0.766 | 0.060 | 0.328 |
| ATTD CP, %[6] | 78.69 | 77.00 | 75.43 | 81.78 | 79.12 | 1.127 | 0.048 | 0.963 | < 0.001 | 0.026 |

[1] A total of 981 pigs (initially 10.3 ± 0.19 kg) were used in a 21-d growth study with 19 to 20 pigs per pen and 10 replicates per treatment.

[2] DAP = diammonium phosphate.

[3] Comparing the main effects of low, moderate, and high feed-grade AA inclusion without added N.

[4] Pairwise comparison between high feed-grade AA + DAP and high feed-grade AA.

[5] Pairwise comparison between high feed-grade AA + glycine and high feed-grade AA.

[6] DAP to moderate feed-grade AA ($P = 0.005$).

**Table 1.11.** Interactive effects of standardized ileal digestible (SID) Lys to crude protein (CP) and SID Lys on growth performance (Exp. 2)[1]

| | SID Lys, % | | | | | | | | | | | *P* = | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1.15 | | | | | 1.30 | | | | | | SID Lys:CP × SID Lys | | |
| SID Lys:CP: | 6.00 | 6.22 | 6.46 | 6.72 | 7.00 | 6.00 | 6.22 | 6.46 | 6.72 | 7.00 | SEM | Linear | Quadratic | SID Lys[2] |
| BW, kg | | | | | | | | | | | | | | |
| d 0 | 13.0 | 13.0 | 13.0 | 12.9 | 13.0 | 12.9 | 13.2 | 13.0 | 13.1 | 13.0 | 0.27 | 0.474 | 0.207 | 0.230 |
| d 14 | 21.3 | 21.2 | 21.2 | 21.1 | 21.1 | 21.3 | 21.9 | 21.6 | 21.6 | 21.5 | 0.40 | 0.602 | 0.253 | 0.002 |
| Overall (d 0 to 14) | | | | | | | | | | | | | | |
| ADG, g | 588 | 582 | 588 | 586 | 579 | 600 | 617 | 609 | 601 | 604 | 12.3 | 0.917 | 0.720 | < 0.001 |
| ADFI, g | 855 | 851 | 862 | 884 | 894 | 846 | 866 | 862 | 867 | 866 | 21.4 | 0.165 | 0.319 | 0.291 |
| G:F, g/kg[3] | 689 | 684 | 683 | 664 | 649 | 709 | 713 | 708 | 695 | 698 | 9.8 | < 0.001 | 0.133 | < 0.001 |

[1] A total of 4,167 pigs (initially 13.0 ± 0.27 kg) were used in a 14-d growth study with 26 pigs per pen (52 pigs per feeder) and eight replications per treatment.

[2] Main effect of SID Lys.

[3] Quadratic effect of SID Lys:CP in 1.15% SID Lys, $P = 0.016$. Quadratic effect of SID Lys:CP in 1.30% SID Lys, $P = 0.777$; Linear effect of SID Lys:CP in 1.15% SID Lys, $P < 0.001$. Linear effect of SID Lys:CP in 1.30% SID Lys, $P < 0.001$.

**Table 1.12.** Main effects of standardized ileal digestible (SID) Lys to crude protein (CP) on growth performance (Exp. 2)[1]

| | SID Lys:CP | | | | | | *P* = | |
|---|---|---|---|---|---|---|---|---|
| | 6.00 | 6.22 | 6.46 | 6.72 | 7.00 | SEM | Linear | Quadratic |
| BW, kg | | | | | | | | |
| d 0 | 13.0 | 13.1 | 13.0 | 13.0 | 13.0 | 0.25 | 0.944 | 0.704 |
| d 14 | 21.3 | 21.5 | 21.4 | 21.3 | 21.3 | 0.38 | 0.731 | 0.418 |
| Overall (d 0 to 14) | | | | | | | | |
| ADG, g | 594 | 599 | 598 | 594 | 591 | 10.9 | 0.502 | 0.397 |
| ADFI, g | 851 | 859 | 862 | 875 | 880 | 19.7 | 0.004 | 0.953 |
| G:F, g/kg | 699 | 698 | 695 | 679 | 673 | 9.5 | < 0.001 | 0.056 |

[1] A total of 4,167 pigs (initially 13.0 ± 0.27 kg) were used in a 14-d growth study with approximately 26 pigs per pen (52 pigs per feeder).

**Table 1.13.** Interactive effects of standardized ileal digestible (SID) Lys to crude protein (CP) and dried distillers grains with solubles (DDGS) on growth performance (Exp. 3)[1]

| | No DDGS | | | | | | 15% DDGS | | | | | | | SID Lys:CP × DDGS, $P$ = | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SID Lys:CP: | 6.01 | 6.22 | 6.45 | 6.70 | 6.97 | 7.26 | 6.01 | 6.22 | 6.45 | 6.70 | 6.97 | 7.26 | SEM | Linear | Quadratic |
| BW, kg | | | | | | | | | | | | | | | |
| d 0 | 10.9 | 11.0 | 11.0 | 11.0 | 10.9 | 11.2 | 11.0 | 11.1 | 11.0 | 10.9 | 11.1 | 10.9 | 0.90 | 0.511 | 0.745 |
| d 18 | 20.1 | 20.4 | 20.4 | 20.6 | 20.6 | 20.7 | 20.1 | 20.2 | 20.7 | 20.4 | 20.4 | 19.9 | 1.28 | 0.267 | 0.464 |
| Overall (d 0 to 18) | | | | | | | | | | | | | | | |
| ADG, g[2] | 506 | 521 | 514 | 529 | 534 | 524 | 499 | 503 | 530 | 517 | 513 | 500 | 22.5 | 0.282 | 0.358 |
| ADFI, g | 742 | 748 | 732 | 744 | 763 | 760 | 720 | 722 | 752 | 748 | 756 | 751 | 45.3 | 0.540 | 0.219 |
| G:F, g/kg[3,4] | 683 | 699 | 704 | 713 | 702 | 692 | 696 | 700 | 708 | 694 | 682 | 669 | 13.6 | < 0.001 | 0.437 |

[1] A total of 5,059 pigs (initially 11.0 ± 0.90 kg) were used in an 18-d growth study with approximately 35 pigs per pen and 11 or 12 replications per treatment.

[2,3] Main effect of DDGS, [2]$P = 0.071$. [3]$P = 0.010$.

[4] Quadratic effect of SID Lys:CP in no DDGS, $P < 0.001$. Linear ($P < 0.001$) and quadratic ($P = 0.002$) effect of SID Lys:CP in 15% DDGS diets.

**Table 1.14.** Main effects of standardized ileal digestible (SID) Lys to crude protein (CP) on growth performance (Exp. 3)[1]

| | SID Lys:CP | | | | | | | *P* = | |
|---|---|---|---|---|---|---|---|---|---|
| | 6.01 | 6.22 | 6.45 | 6.70 | 6.97 | 7.26 | SEM | Linear | Quadratic |
| BW, kg | | | | | | | | | |
| d 0 | 11.0 | 11.0 | 11.0 | 11.0 | 11.0 | 11.0 | 0.89 | 0.895 | 0.971 |
| d 18 | 20.1 | 20.3 | 20.5 | 20.5 | 20.5 | 20.3 | 1.26 | 0.432 | 0.145 |
| Overall (d 0 to 18) | | | | | | | | | |
| ADG, g | 503 | 512 | 522 | 523 | 524 | 512 | 21.3 | 0.211 | 0.021 |
| ADFI, g | 731 | 735 | 742 | 746 | 760 | 755 | 44.0 | 0.018 | 0.714 |
| G:F, g/kg | 690 | 699 | 706 | 703 | 692 | 680 | 13.1 | 0.007 | < 0.001 |

[1] A total of 5,059 pigs (initially 11.0 ± 0.90 kg) were used in an 18-d growth study with approximately 35 pigs per pen.

# Chapter 2 - Effects of novel specialty protein products on growth performance and fecal dry matter of nursery pigs

## Abstract

Two experiments were conducted evaluating the effects of specialty soy protein products on nursery pig growth performance and fecal dry matter (DM). In Exp. 1, 360 barrows (DNA 200 × 400; initially 5.6 ± 0.02 kg) were used in a 37-d study to assess a refined soy protein concentrate (SPC) as a replacement for soybean meal (SBM) on a standardized ileal digestible (SID) Lys basis. Diets contained increasing SPC (0, 4.25, 8.5, 12.75, and 17%) replacing 0, 25, 50, 75, or 100% of SBM, and a positive control containing 8.5% enzymatically treated SBM (ESBM). Average daily gain (ADG) and gain-to-feed ratio (G:F) for the experimental period (d 0 to 23) increased (quadratic, $P < 0.05$) then decreased as SPC increased, with the greatest improvement observed when SPC replaced up to 50% of the SBM. Additionally, average daily feed intake (ADFI) increased (linear, $P = 0.019$) as SPC increased. Overall (d 0 to 37), increasing SPC increased (quadratic, $P = 0.014$) ADG and tended to increase (quadratic, $P = 0.080$) ADFI with the greatest response at 50% and 75% replacement of SBM, respectively. Gain-to-feed ratio tended to decrease (linear, $P = 0.087$) in pigs previously fed increasing SPC for the first 23 d. Fecal DM increased as SPC increased (linear, $P = 0.035$) on d 9, and pigs fed 50% SBM replacement (8.5% SPC) had greater ($P = 0.011$) fecal DM than those fed 8.5% ESBM. In Exp. 2, 1,254 mixed-sex pigs (PIC 800 × [Fast LW × PIC L02]; initially 5.6 ± 0.10 kg) were used in a 28-d study to evaluate thermo-mechanically processed SBM (TM-SBM) at increasing levels replacing SBM on a SID Lys basis (0, 25, 50, 75, and 100% in phase 1 and 0, 12.5, 25, 37.5, and 50% in phase 2). For the experimental period (d 0 to 21), ADG and ADFI increased then decreased (quadratic, $P \leq 0.038$) with the greatest improvement observed when TM-SBM replaced 25 to 50% of the SBM from d 0 to 7 and 12.5 to 25% from d 7 to 21 with an increase in G:F (quadratic, $P = 0.036$) observed with TM-SBM replacing 50 and 25% of the SBM from d 0 to 7 and d 7 to 21, respectively, but worsening thereafter. A treatment × day interaction was observed for fecal DM (quadratic, $P = 0.024$). On d 7, fecal DM increased when TM-SBM replaced 25% of the SBM (quadratic, $P = 0.004$), but decreased with 50% replacement before remaining constant, with no differences on d 21. Ultimately, replacing 25 to 50% of the digestible Lys provided by SBM in diets for 5 to 13 kg pigs with specialty soy products improved growth performance and fecal DM.

**Key Words:** fecal dry matter, nursery pig, soy protein concentrate, specialty soy protein, thermo-mechanically processed soybean meal

## Introduction

Weaning is a stressful period for pigs where they experience physiological, environmental, and social changes (Campbell et al., 2013). One of the most significant challenges is the sudden change in diet from a highly digestible, liquid milk-based diet to a solid, plant-based diet usually comprised of corn and soybean meal (SBM). Soybean meal is an economical protein source for use in swine diets, but it needs to be gradually introduced into diets of weanling pigs. This is due to the presence of complex carbohydrates, like stachyose and raffinose, as well as trypsin inhibitors, antigens, and indigestible oligosaccharide storge proteins like glycinin and β-conglycinin (Zheng et al., 2021). These components found in SBM can cause hypersensitivity in nursery pigs (Hao et al., 2009), which may negatively affect growth performance and increase the risk of post-weaning diarrhea. Weanling pigs also experience low feed intake, which worsens post-weaning challenges (Le Dividich and Sève, 2000). Therefore, incorporating protein sources with lower anti-nutritional factors (ANF) and higher digestibility is important to encourage feed intake in weaned pigs.

Further processing of soy products can improve digestibility and tolerance in weanling pigs compared to conventional SBM (Pedersen et al., 2016). However, specialty soy products can vary in nutrient profile, levels of complex carbohydrates, antinutritional factors, and palatability. Several refined SBM-based products have been developed to mitigate limitations such as ANF in nursery pig diets, including enzymatically treated SBM (Long et al., 2021), soy protein concentrate (Deng et al., 2022), fermented SBM (Yuan et al., 2016), and other specialty soy proteins. Each processing method affects protein quality, digestibility, palatability, and nutritional composition differently.

Soy protein concentrate is produced by removing soluble carbohydrates and other non-protein components from defatted soy flakes through ethanol extraction, resulting in a product that contains approximately 65 to 70% protein (Peisker, 2001). Research using soy protein concentrates has shown variable results, with some reporting a positive effect on growth performance when replacing animal protein supplements (Deng et al., 2023), while others have reported negative effects on growth performance compared to animal protein products (Nofrarías et al., 2007; Che et al., 2012). A specific refined soy protein concentrate (SPC; PurePro Soy, Bunge North America Inc., Chesterfield, MO) that is commercially available for use in diets has reduced levels of complex carbohydrates like stachyose (1.4%) and raffinose (0.2%). Whereas conventional SBM can contain approximately 5.2% stachyose and 1.1% raffinose (Cervantes-Pahm and Stein, 2010). Additionally, it has lower concentrations of glycinin and β-conglycinin

(< 5% and 2%, respectively) compared to conventional SBM, which can have concentrations of approximately 14.3% glycinin and 10.1% β-conglycinin (Park et al., 2020). With reduced ANF, increased quantities of specialty processed soy protein may be included in weanling pig diets as replacement for other specialty protein sources.

Another further processed specialty soy protein product has recently become available and is produced by thermo-mechanical processing of SBM (TM-SBM; Provisoy, Cargill Animal Nutrition, Minneapolis, MN). This additional processing step reduces ANF, such as trypsin inhibitors, compared to conventional SBM. This specific thermo-mechanically processed SBM has been shown to support gut health (Hu et al., 2023a) and improve protein digestibility (Hu et al., 2023b) in *Escherichia coli* challenged pigs. However, this product has not been evaluated in a non-challenged, commercial setting.

Limited data is available that describes the optimum dietary addition of these specialty protein sources in diets for newly weaned pigs. Therefore, the objective of these two experiments was to evaluate the effects of replacing SBM with specialty soy proteins on nursery pig growth performance and fecal dry matter. It was hypothesized that partially replacing SBM with specialty soy products would improve nursery pig growth performance and increase fecal dry matter.

## Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments (IACUC #4506.19 and #4892). Nutrient profiles for the specialty soy protein products tested were obtained from the manufacturers for diet formulation. Samples of the products were analyzed at the University of Missouri Agricultural Experiment

Station Chemical Laboratory for CP (Method 990.03; AOAC International, 2006), ash, dry matter (DM; Method 935.29; AOAC International, 2006), ether extract (ANKOM Technology, 2004), crude fiber (ANKOM Technology, 2005), and complete AA profile (Method 982.30; AOAC International, 2006; Table 1).

## Experiment 1

Experiment 1 was conducted at the Kansas State University Segregated Early Weaning facility in Manhattan, KS. The facility has two identical barns that are completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contained a 4-hole, dry self-feeder and a cup waterer for ad libitum access to feed and water. Pens (1.2 × 1.2 m) had metal tri-bar floors and allowed approximately 0.25 $m^2$/pig.

A total of 360 barrows (DNA 200 × 400; initially 5.6 ± 0.02 kg) were used in a 37-d growth trial. Pigs were weaned at approximately 21 d of age and blocked by initial weight. Pens were assigned in a generalized randomized block design to one of six dietary treatments. Pigs were blocked into light (initially 5.0 ± 0.01 kg) and heavy groups (initially 6.2 ± 0.01 kg). There were five pigs per pen, and within each block, there were six pens per treatment (three pens per weight group in each barn) for a total of 12 replications per treatment. Diets were corn-soybean meal-based and consisted of increasing SPC (0, 4.25, 8.5, 12.75, and 17%) replacing 0, 25, 50, 75, or 100% of the standardized ileal digestible (SID) Lys provided by SBM in the diet, respectively. A sixth diet served as a positive control containing 8.5% enzymatically treated SBM (HP 300, Hamlet Protein; Findlay, OH) replacing a portion of the SBM in the diet (Tables 2 and 3). All diets were formulated to the same SID Lys level and were similar in energy content with SBM net energy (NE) considered to be 92% of corn NE. Dietary additions of feed-grade amino acids (AA) were adjusted to meet or exceed AA requirements in relation to Lys for Ile, Met and Cys, Thr, Trp, and Val. Pigs were fed treatment diets in two phases with phase 1 from d 0 to 9, followed by phase 2 from d 9 to 23. Subsequently, all pigs were fed a common corn-soybean meal-based diet without specialty soy protein sources until d 37 of the trial.

Treatment diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS and fed in pellet form for phase 1 and meal form in phases 2 and 3. Pig weights and feed disappearance were measured on d 0, 9, 16, 23, 30, and 37 to determine average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F). Feces were collected at the end of phases 1 (d 9) and 2 (d 23) from the same three randomly selected pigs in each pen for dry matter (DM) analysis. Fecal samples were dried at 55°C in a forced air oven for 48 h, and the ratio of dried to wet fecal weight determined the percentage fecal dry matter. Fecal samples were analyzed separately for each pig, and the average of the three samples from each pen was then used for statistical analysis.

### Experiment 2

Experiment 2 was conducted at the New Fashion Pork (Jackson, MN) research wean-to-finish facility to evaluate the effect of a specialty TM-SBM in a commercial research setting. The facility consisted of two rooms with a total of 62 pens. The facility was completely enclosed, environmentally regulated, and mechanically ventilated. Each pen contained a three-hole, dry self-feeder and a bowl waterer for ad libitum access to feed and water.

A total of 1,254 mixed-sex pigs (PIC 800 × [Fast LW × PIC L02]; initially 5.6 ± 0.10 kg) were used in a 28-d growth study. Pens of pigs were randomly allotted to one of five dietary treatments in a randomized complete block design with initial body weight (BW) and nursery entry date as blocking factors. There were 18 to 20 pigs per pen and 12 pens per treatment. The experimental diets were corn-soybean meal-based with increasing TM-SBM (0, 5.79, 11.58, 17.36, and 23.02%) replacing 0, 25, 50, 75, and 100% of the SID Lys provided by SBM in phase 1 and 0, 3.21, 6.41, 9.62, and 12.82% replacing 0, 12.5, 25, 37.5, and 50% in phase 2 (Table 4). All diets were formulated to the same SID Lys level. The same amount of L-Lys HCl was used in each diet within phase, but additions of other feed-grade AA were adjusted to equalize Met and Cys, Thr, Trp, and Val concentrations relative to Lys in all diets. Nutrient loading values for TM-SBM were provided by the supplier and values for the other ingredients were obtained from the NRC (2012). Like Exp. 1, treatment diets were fed in two phases but with a feed budget of 2.7 kg per pig for phase 1 (d 0 to 7) and 4.5 kg for phase 2 (d 7 to 21) followed by a common diet from d 21 to 28 (phase 3).

All diets were fed in meal form and were manufactured at the New Vision CO-OP (Worthington, Minnesota). Pig weights and feed disappearance were measured on d 0, 7, 14, 21, and 28 to determine ADG, ADFI, and G:F. Fecal samples were also collected at the end of phases 1 (d 7) and 2 (d 21) from three randomly selected pigs per pen to determine percentage DM. Fecal samples were processed and analyzed using the same procedure as Exp. 1. In addition, the feces that were collected for fecal DM were also scored on a 0 to 4 scoring system: 0 = hard, pellet-like lumps; 1 = firm, formed feces; 2 = normal feces; 3 = mild looseness; and 4 = diarrhea by two observers.

## Statistical Analysis

### Experiment 1

Data were analyzed as a generalized randomized block design as a one-way ANOVA using the lmer function from the lme4 package in R Studio (Version 4.3.1, R Core Team. Vienna, Austria) with pen serving as the experimental unit and dietary treatment, weight block, and the associated interaction as fixed effects. Barn was included in the model as a random effect. Linear and quadratic contrasts were tested within increasing levels of SPC, excluding the ESBM treatment. The effect of 8.5% ESBM was tested by a pairwise comparison with 8.5% SPC (50% replacement of SBM). Fecal DM samples were analyzed using the fixed effects of day, treatment, block, and the associated interactions accounting for repeated measures over time.

### Experiment 2

Data were analyzed as a randomized complete block design as a one-way ANOVA using the lmer function from the lme4 package in R Studio (Version 4.3.1, R Core Team. Vienna, Austria) with pen serving as the experimental unit, dietary treatment as a fixed effect, and weight block as a random intercept. Linear and quadratic contrasts were used to test for increasing levels of TM-SBM. Fecal DM samples were analyzed using the fixed effects of day, treatment, and the associated interactions accounting for repeated measures over time. Fecal scores were summarized using the FREQ procedure of SAS OnDemand for Academics (SAS Institute, Inc., Cary, NC) and reported as a percentage of observations within each score category by treatment. Fecal score data were analyzed as a categorical variable using the GLIMMIX procedure of SAS. Results for both experiments were considered significant with $P \leq 0.05$ and were considered marginally significant with $0.05 < P \leq 0.10$.

## Results

### Experiment 1

There were no significant interactions of treatment and BW block, therefore interpretation is focused on the main effect of treatment. From d 0 to 9 (phase 1), a tendency for an improvement in ADG (quadratic, $P = 0.077$) was observed as SPC replaced up to 50% of SBM resulting in a tendency for an increase (quadratic, $P = 0.052$) in d 9 BW (Table 5).

Additionally, as SPC increased, G:F increased (quadratic, $P = 0.029$) with the greatest improvement at 50% SBM SID Lys replacement. When comparing 50% SPC replacement (8.5% inclusion) to 8.5% ESBM, there was a tendency for improvement in G:F for pigs fed SPC ($P = 0.065$). From d 9 to 23 (phase 2), ADG tended to increase (quadratic, $P = 0.072$) and d 23 BW increased (quadratic, $P = 0.030$) with increasing SPC, with the greatest improvement observed at 50% replacement of SBM in the diet. Additionally, as SPC increased up to 75% SBM replacement, ADFI increased (linear, $P = 0.024$). This resulted in decreased G:F as SPC replacement increased up to 100% of the SBM in the diet (linear, $P = 0.003$). There were no treatment differences between 50% SPC replacement (8.5% inclusion) and 8.5% ESBM. For the experimental period (d 0 to 23), ADFI increased (linear, $P = 0.019$) as SPC increased. Additionally, ADG and G:F increased (quadratic, $P \leq 0.030$) with the maximum response at 50% replacement of SBM. In the common period (phase 3), no differences in ADG, ADFI, or G:F were observed due to the inclusion of SPC in the previous diets. However, d 37 BW increased (quadratic, $P = 0.035$) as SPC previously increased up to a replacement of 50% SBM. No treatment differences were observed between 50% SPC replacement (8.5% inclusion) and 8.5% ESBM.

Overall (d 0 to 37), increasing SPC increased ADG (quadratic, $P = 0.014$) with the greatest improvement at 50% SBM replacement and tended to decrease (linear, $P = 0.087$) G:F up to 100% SPC replacement of SBM. Additionally, ADFI tended to increase (quadratic, $P = 0.080$) up to 50% SPC replacement of SBM in the diet. There were no treatment differences between 50% SPC replacement (8.5% inclusion) and 8.5% ESBM.

Pigs fed increasing SPC had increased fecal DM on d 9 (linear, $P = 0.035$), and those fed 50% SPC replacement of SBM (8.5% inclusion) had greater ($P = 0.011$) fecal DM than pigs fed ESBM. There were no differences in fecal DM on d 23.

### Experiment 2

From d 0 to 7 (phase 1), ADG, ADFI, and d 7 BW increased (quadratic, $P \leq 0.003$) with increasing TM-SBM up to 50% of the SBM replacement and decreased thereafter (Table 6). Additionally, G:F increased (quadratic, $P < 0.001$) as TM-SBM replacement of SBM increased up to 75% and worsened at 100% replacement of SBM. From d 7 to 21 (phase 2), ADG decreased (linear, $P = 0.029$) and G:F tended to decrease (linear, $P = 0.072$) with increasing TM-SBM. Day 21 BW increased then decreased (quadratic, $P = 0.020$) with increasing TM-SBM, which was a result of pigs being the heaviest when 25 to 50% of SBM was replaced in phase 1 followed by 12.5 to 25% replacement in phase 2. From d 0 to 21 (experimental period), ADG and ADFI increased then decreased (quadratic, $P \leq 0.038$) with the greatest gain and feed intake observed when TM-SBM replaced 25 to 50% of the SBM in phase 1 and 12.5 to 25% of the SBM in phases 2. Gain-to-feed ratio increased (quadratic, $P = 0.036$) when TM-SBM replaced up to 50% of SBM in phase 1 and 25% in phase 2. From d 21 to 28 (common period), no significant differences were observed in ADG, ADFI, or G:F. Overall (d 0 to 28), ADG and ADFI tended to increase up to the treatment with 25 and 12.5% SBM replacement in phases 1 and 2, respectively, with performance decreasing (quadratic, $P \leq 0.089$) with further replacement of SBM.

A significant treatment × day interaction was observed for fecal DM (quadratic, $P = 0.024$) driven by a treatment effect on d 7 but no treatment differences observed on d 21. On d 7, fecal DM increased as TM-SBM increased from 0 to 25% replacement of SBM (quadratic, $P =$

0.004), but then decreased at the 50% replacement level and remaining relatively consistent thereafter. Similar to fecal DM, a treatment × day interaction was observed for fecal scoring (quadratic, $P = 0.017$) driven by a treatment effect on d 7 (quadratic, $P < 0.001$) with the greatest reduction in diarrhea incidence occurring when pigs were fed diets with TM-SBM replacing 25% of the SBM (Figure 1); however, no treatment difference in fecal score was observed on d 21.

Overall, there were no observed treatment differences in mortality; however, the percentage of removals, as well as the combined total of removals and mortality, decreased (linear, $P \leq 0.014$) as the inclusion of TM-SBM increased from d 0 to 21.

## Discussion

Dehulled SBM is a common protein source, containing approximately 47.5% CP (NRC, 2012). Additional processing can enhance SBM's CP content while reducing ANF that may decrease weanling pig growth. Two methods of further processing SBM include enzymatic treatment (EBSM) and thermo-mechanical processing (TM-SBM). Enzyme treatment of SBM involves the use of a specialized blend of enzymes (proteases, carbohydrases, and sometimes phytases) to break down carbohydrates and reduce ANF (Cervantes-Pahm and Stein, 2010). Alternatively, thermo-mechanically processed SBM is a method used to enhance the nutritional quality of SBM by applying heat, pressure, and shear force. The heat treatment denatures ANF like trypsin inhibitors and allergenic proteins, which can improve digestibility and nutrient absorption (Ton Nu et al., 2020). In contrast, soy protein concentrate is obtained by extracting water-soluble carbohydrates from defatted soy flakes using aqueous ethanol while retaining most of the insoluble fiber, resulting in a product that contains a minimum of 65% CP (Wang et al., 2004).

Lenehan et al. (2007) observed that pigs fed diets containing a soy protein concentrate product included at 14.3% exhibited greater ADG compared to those fed 28.6% where SBM was completely replaced, suggesting that a high inclusion of specialty soy protein can negatively affect growth performance. This was also observed in both of the current experiments where total replacement of SBM with specialty soy products led to a reduction in growth performance relative to lower replacement levels. In a second study, Lenehan et al. (2007) observed that as soy protein concentrate increased and replaced SBM, ADG increased up to 14.3% SPC (50% replacement of SBM). Also, ADFI increased quadratically as soy protein concentrate inclusion increased to 21.4% (75% replacement of SBM). Similar results were observed in Exp. 1, where ADG increased with SPC inclusion up to 50% SBM replacement (8.5% inclusion) before decreasing with further replacement, while ADFI tended to follow a quadratic increase up to 75% SBM replacement. Likewise, this response was observed in Exp. 2, where ADG exhibited a quadratic trend, peaking at 50% and 25% TM-SBM replacement for SBM in phases 1 and 2, respectively. However, in contrast to Lenehan et al. (2007) and Exp. 1, ADFI was highest at 25% replacement in phase 1 and 12.5% in phase 2 and decreased with further replacement of SBM with TM-SBM. This may be due to the differences in processing methods as TM-SBM does not remove carbohydrates, unlike SPC, and high levels of TM-SBM may result in an allergic response to the complex carbohydrates. Overall, these data suggest that 100% replacement of SBM in nursery diets with specialty soy products can negatively affect growth performance.

The poorer performance of pigs fed the control diet in both of the current experiments may be attributed to hypersensitivity to SBM, as ADFI was low for these treatments. Glycinin and β-conglycinin are two major storage proteins in SBM, which account for approximately 65 to 80% of the total protein content (Thrane et al., 2012). These proteins have been shown to induce transient hypersensitivity in weaned pigs, leading to cellular-level abnormalities in the gastrointestinal tract, such as reduced villous height and crypt hypertrophy, which can negatively affect growth performance (Chen et al., 2011). This response may also increase susceptibility to disease challenges and reduce overall resiliency during the post-weaning period. In Exp. 2, pigs fed the control diet with no TM-SBM not only had reduced feed intake but also had a higher percentage of pigs removed from the study due to poor growth and losing weight, suggesting compromised health status associated with SBM sensitivity.

Lenehan et al. (2007) investigated the palatability of soy protein concentrate in nursery pig diets and observed a preference for a diet containing 40% SBM over a diet where soy protein concentrate completely replaced SBM (28.6% inclusion). The product used by Lenehan et al. (2007) was an aqueous alcohol-extracted SPC that undergoes moist extrusion, which may have contributed to the differences in palatability. Additionally, Solà-Oriol et al. (2011) observed that pigs preferred soy protein concentrate when added at 20% of the diet compared to a control diet containing 20% ESBM. However, in Exp. 1, no differences in feed intake were observed between pigs fed SPC and those fed ESBM. While high SPC replacement of SBM did not appear to affect feed intake during the experimental period in Exp. 1, fully replacing SBM with 17% SPC was associated with decreased growth performance.

A possible explanation for the reduction in growth performance at 100% replacement of SBM with 17% SPC may be a deficiency of non-essential amino acids (NEAA) due to the lower SID Lys to dietary crude protein ratio (SID Lys:CP) when SPC completely replaced SBM in the diet. The NRC (2012) estimates a minimum of 3.63 and 3.29% total dietary N for pigs weighing 5 to 7 kg and 7 to 11 kg, respectively, along with SID Lys levels of 1.50 and 1.35%, respectively. Based on these values, the maximum SID Lys:N ratio can be estimated, or total N can be converted to a CP basis using a factor of 6.25 to obtain the SID Lys:CP ratio. Based on NRC (2012) estimates, diets should be formulated to maintain a SID Lys:CP ratio below 6.61% for 5 to 7 kg pigs and 6.57% for 7 to 11 kg pigs. In both experiments, the diets with 100% SBM replacement resulted in SID Lys:CP ratios that exceeded the estimate (6.93 and 7.00% in phases 1 and 2, respectively, for Exp. 1 and 6.75 and 6.72% in phases 1 and 2, respectively, for Exp. 2). This is because of the greater SID Lys:CP ratio of the specialty protein products compared to SBM. Previous research (Smallfield et al., 2025a,b) suggests that G:F decreases when the SID Lys:CP ratio exceeds the estimated SID Lys:CP ratio, which was observed in the studies herein.

Numerous studies have evaluated the effects of enzymatically treated SBM as a partial replacement for SBM in nursery pig diets. Jones et al. (2017) fed diets ranging from 6.67 to 20.00% ESBM in phase 1 and 5.00 to 15.00% in phase 2 and observed that pigs fed the lowest inclusion (6.67 and 5.00% in phases 1 and 2, respectively) had improved growth performance during the experimental period compared to pigs fed the higher levels. The reduction in ADG was primarily driven by decreased ADFI, possibly due to palatability issues, as feed intake decreased with increasing enzymatically treated SBM. The bitter taste associated with high enzymatically treated SBM additions is thought to result from proteolytic enzymes exposing the hydrophobic AA located within the interior of the protein (Liu et al., 2022).

The ideal level of enzymatically treated SBM in weanling pig diets varies across studies. Tan et al. (2024) found 4% enzymatically treated SBM to be optimal compared to either 2 or 8%. Ruckman et al. (2020) reported 7% ESBM as the ideal inclusion for growth compared to 14 or 21%. However, the enzymatically treated SBM used by Tan et al. (2024) was different than the source used in our study. Because the previous research suggested that high amounts of enzymatically treated SBM can decrease growth performance, 8.5% ESBM was used to compare with 8.5% SPC (50% SBM replacement) in Exp. 1. Yang et al. (2007) compared pigs fed nursery diets containing 8% soy protein concentrate and 8% ESBM and reported that pigs fed soy protein concentrate had greater ADG than those fed ESBM during the first two weeks post-weaning. This observed advantage in growth performance with SPC inclusion contrasts with the results observed in Exp. 1 where there were no treatment differences observed between pigs fed diets containing 8.5% SPC and those fed 8.5% ESBM. However, this may be due to Yang et al. (2007) using a soy protein concentrate source other than the one used in the current study.

The linear increase in fecal DM with increasing SPC on d 9 may be associated with the decreasing CP content of the diet as SPC increased. A low CP diet reduces the availability of substrate for bacteria fermentation, thereby improving fecal consistency (Pieper et al., 2016). As pigs age and their digestive tract matures, they become less susceptible to the effects of SBM hypersensitivity, intestinal barrier dysfunction, and pathogenic growth (Han et al., 2024). This likely explains the lack of treatment differences in fecal DM in both experiments at the end of phase 2. Fecal DM was lowest with pigs fed the control diet with no specialty soy protein in both experiments, which could be attributed to the greater concentration of glycinin, β-conglycinin, and trypsin inhibitor in SBM. These anti-nutritional factors have been suggested to cause damage to intestinal morphology and cause an allergic reaction and diarrhea in weanling pigs (Li et al., 1991). Overall values for fecal DM were lower in Exp. 2 than that observed in Exp. 1. This might be explained by environment where Exp. 2 was conducted in a commercial research facility while Exp. 1 took place in a university setting. Montagne et al. (2012) observed that pigs raised in an environment with greater microbial pressure had lower fecal DM compared to those in a cleaner environment.

Fecal scores on d 7 exhibited a quadratic response with increasing TM-SBM, where pigs fed the control diet and those fed the highest level of TM-SBM had the greatest frequency of diarrhea. Fecal scores are commonly used in the industry as they provide a simple method for assessing the incidence of diarrhea, though they can be more subjective compared to measuring fecal DM. However, these findings suggest that fecal DM measurements and fecal scoring provided consistent trends.

Although there are a few studies evaluating the effects on fecal consistency when replacing SBM with SPC, more research has compared SPC to other protein sources. Deng et al. (2022) found no differences in fecal consistency across varying levels of soy protein concentrate replacement of animal protein supplements (0, 33, 66, and 100%), despite observing differences in growth performance. Similarly, Wen et al. (2017) reported no difference in fecal scores between pigs fed a soy protein concentrate with 19% CP and soy protein concentrate with 23% CP. This finding is particularly interesting, given that previous research suggests high CP diets can increase the incidence of diarrhea (Limbach et al., 2021). However, Wen et al. (2017) observed that pigs fed soy protein concentrates had less diarrhea than those fed a control diet formulated to 17% CP. In contrast, Ma et al. (2019) observed that pigs fed a SBM-based control diet without processed soy products had a greater incidence of diarrhea than those fed 7.51% soy protein concentrate during the first two weeks post-weaning. This was similarly observed in both experiments where the diets with no specialty soy products had the lowest fecal DM after phase 1.

In summary, these data suggest that replacing SBM with 25 to 50% SPC or TM-SBM in phase 1 diets improves nursery pig growth performance and fecal DM. Furthermore, 50% SPC replacement of SBM (8.5% inclusion) resulted in similar growth performance to ESBM, while improving fecal DM. Fecal scoring results align with trends observed in fecal DM, suggesting that either method can be utilized. Overall, complete replacement of SBM with specialty soy products is not recommended, as higher additions of SPC and TM-SBM were associated with poorer growth performance.

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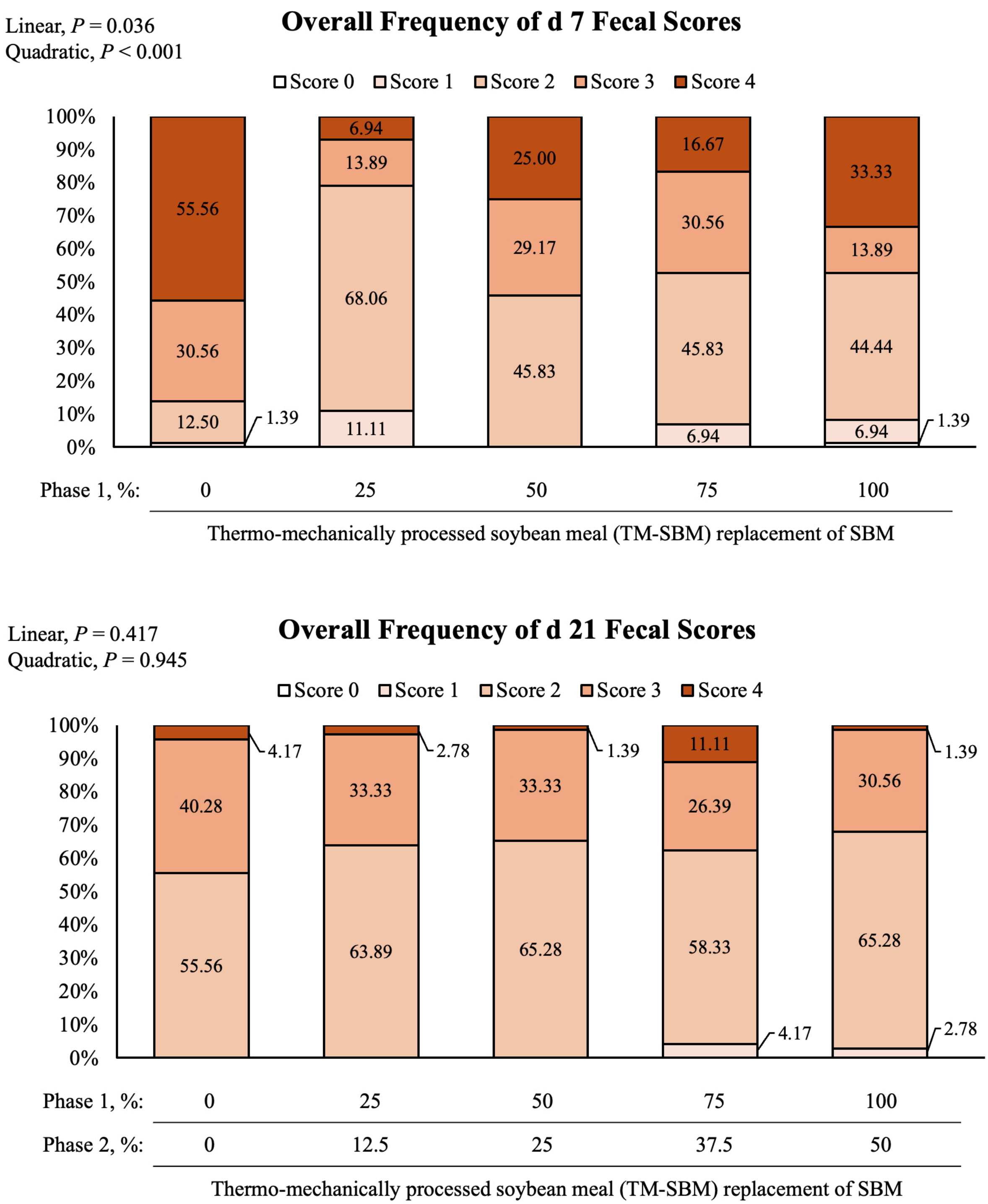


**Figure 2.1.** Effect of thermo-mechanically processed soybean meal (TM-SBM) replacement of SBM on fecal score frequency (Exp. 2). Fecal scores are presented on a 4-point scale: 0 = hard, pellet-like lumps; 1 = firm, formed feces; 2 = normal feces; 3 = mild looseness; and 4 = diarrhea scored by two observers on d 7 and 21. Treatment × day interaction (quadratic, $P = 0.017$). Main effect of treatment (linear, $P = 0.041$; quadratic, $P = 0.014$) and day ($P = 0.004$).

**Table 2.1.** Analyzed composition of specialty soy protein sources for Exp. 1 and 2 (as-fed basis)[1]

| | Specialty soy protein | |
|---|---|---|
| Nutrient, % | Soy protein concentrate (SPC)[2] | Thermo-mechanically processed soybean meal (TM-SBM)[3] |
| CP | 66.98 | 48.57 |
| Dry matter | 93.12 | 93.30 |
| Crude fat | 0.14 | 1.43 |
| Crude fiber | 3.94 | 3.92 |
| Ash | 5.42 | 6.81 |
| Essential AA | | |
| Arg | 4.82 | 3.46 |
| His | 1.78 | 1.27 |
| Ile | 3.34 | 2.38 |
| Leu | 5.30 | 3.73 |
| Lys | 4.34 | 3.13 |
| Met | 0.91 | 0.67 |
| Phe | 3.46 | 2.50 |
| Thr | 2.56 | 1.86 |
| Trp | 0.94 | 0.68 |
| Val | 3.50 | 2.47 |
| Non-essential AA | | |
| Ala | 2.91 | 2.10 |
| Asp | 7.43 | 5.36 |
| Cys | 0.94 | 0.76 |
| Glu | 12.58 | 8.88 |
| Gly | 2.82 | 2.05 |
| Pro | 3.48 | 2.49 |
| Ser | 2.73 | 2.00 |
| Tyr | 2.36 | 1.83 |

[1] Samples were analyzed for proximate analysis and complete AA profile (University of Missouri Agricultural Experiment Station Chemical Laboratory).

[2] PurePro Soy; Bunge; Chesterfield, MO.

[3] Provisoy; Cargill Animal Nutrition; Minneapolis, MN.

**Table 2.2.** Ingredient composition of Exp. 1 diets (as-fed basis)[1]

| | Phase 1 | | | | | |
|---|---|---|---|---|---|---|
| | SPC replacement of SBM, % | | | | | ESBM, % |
| | 0 | 25 | 50 | 75 | 100 | 8.50 |
| Ingredient, % | | | | | | |
| Corn | 43.02 | 46.20 | 49.41 | 52.63 | 55.88 | 44.55 |
| Soybean meal (47% CP) | 30.00 | 22.51 | 15.02 | 7.52 | --- | 19.98 |
| Soy protein concentrate[2] | --- | 4.25 | 8.50 | 12.75 | 17.00 | --- |
| Enzymatically treated SBM[3] | --- | --- | --- | --- | --- | 8.50 |
| Whey powder | 22.50 | 22.50 | 22.50 | 22.50 | 22.50 | 22.50 |
| Soybean oil | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Calcium carbonate | 0.65 | 0.65 | 0.65 | 0.65 | 0.65 | 0.65 |
| Monocalcium P (21% P) | 0.68 | 0.73 | 0.75 | 0.78 | 0.80 | 0.70 |
| Salt | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| L-Lys-HCl | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 |
| DL-Met | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 |
| L-Thr | 0.18 | 0.19 | 0.19 | 0.20 | 0.20 | 0.18 |
| L-Trp | 0.03 | 0.04 | 0.05 | 0.05 | 0.06 | 0.03 |
| L-Val | 0.12 | 0.12 | 0.11 | 0.11 | 0.10 | 0.09 |
| Zinc oxide | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 |
| Vitamin premix | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Trace mineral premix | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Choline chloride | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Phytase[4] | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 |
| Calculated analysis | | | | | | |
| SID AA, % | | | | | | |
| Lys, % | 1.35 | 1.35 | 1.35 | 1.35 | 1.35 | 1.35 |
| Ile:Lys | 59 | 59 | 59 | 59 | 59 | 61 |
| Leu:Lys | 114 | 115 | 116 | 117 | 117 | 116 |
| Met:Lys | 37 | 37 | 37 | 37 | 38 | 36 |
| Met and Cys:Lys | 58 | 58 | 58 | 58 | 58 | 58 |
| Thr:Lys | 65 | 65 | 65 | 65 | 65 | 65 |
| Trp:Lys | 20.0 | 20.1 | 20.2 | 20.2 | 20.1 | 20.0 |
| Val:Lys | 70 | 70 | 70 | 70 | 70 | 70 |
| His:Lys | 35 | 34 | 34 | 33 | 33 | 35 |
| NE, kcal/kg | 2,606 | 2,604 | 2,604 | 2,604 | 2,604 | 2,590 |
| SID Lys:NE, g/Mcal | 5.18 | 5.18 | 5.18 | 5.18 | 5.18 | 5.21 |
| CP, % | 20.8 | 20.5 | 20.2 | 19.8 | 19.4 | 21.0 |
| Ca, % | 0.69 | 0.69 | 0.69 | 0.69 | 0.68 | 0.69 |
| STTD P, % | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.56 |
| Ca:P | 1.11 | 1.11 | 1.11 | 1.11 | 1.12 | 1.11 |

[1] Phase 1 diets were fed from d 0 to 9 (5.6 to 6.8 kg).

[2] SPC; soy protein concentrate; PurePro Soy (Bunge, Chesterfield, MO).

[3] ESBM; enzymatically treated soybean meal; HP 300 (Hamlet Protein, Findlay, OH).

[4] Ronozyme HiPhos (DSM, Parsippany, NJ) included at 2,027 FTU/kg provided an estimated release of 0.14% STTD P.

**Table 2.3.** Ingredient composition of Exp. 1 diets (as-fed basis)[1]

| | Phase 2 | | | | | | Phase 3 |
|---|---|---|---|---|---|---|---|
| | SPC replacement of SBM, % | | | | | ESBM, % | |
| | 0 | 25 | 50 | 75 | 100 | 8.50 | Common |
| Ingredient, % | | | | | | | |
| Corn | 56.13 | 59.32 | 62.53 | 65.74 | 68.95 | 57.61 | 68.06 |
| Soybean meal (47% CP) | 29.99 | 22.50 | 15.00 | 7.51 | --- | 20.01 | 28.12 |
| Soy protein concentrate[2] | --- | 4.25 | 8.50 | 12.75 | 17.00 | --- | --- |
| Enzymatically treated SBM[3] | --- | --- | --- | --- | --- | 8.50 | --- |
| Whey powder | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | --- |
| Calcium carbonate | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 | 0.75 |
| Monocalcium P (21% P) | 0.75 | 0.80 | 0.83 | 0.85 | 0.88 | 0.75 | 0.85 |
| Salt | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.60 |
| L-Lys-HCl | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.55 |
| DL-Met | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.21 |
| L-Thr | 0.24 | 0.24 | 0.24 | 0.25 | 0.25 | 0.24 | 0.23 |
| L-Trp | 0.05 | 0.06 | 0.07 | 0.07 | 0.08 | 0.05 | 0.05 |
| L-Val | 0.15 | 0.15 | 0.15 | 0.14 | 0.14 | 0.15 | 0.16 |
| Zinc oxide | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | --- |
| Vitamin premix | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Trace mineral premix | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Phytase[4] | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.03 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Calculated analysis | | | | | | | |
| SID AA, % | | | | | | | |
| Lys, % | 1.35 | 1.35 | 1.35 | 1.35 | 1.35 | 1.35 | 1.30 |
| Ile:Lys | 55 | 55 | 55 | 55 | 55 | 57 | 53 |
| Leu:Lys | 112 | 113 | 114 | 115 | 115 | 114 | 113 |
| Met:Lys | 37 | 37 | 38 | 38 | 38 | 37 | 36 |
| Met and Cys:Lys | 58 | 58 | 58 | 58 | 58 | 58 | 57 |
| Thr:Lys | 65 | 65 | 65 | 65 | 65 | 65 | 63 |
| Trp:Lys | 20.1 | 20.0 | 20.3 | 20.3 | 20.2 | 20.1 | 19.3 |
| Val:Lys | 70 | 70 | 70 | 70 | 70 | 72 | 70 |
| His:Lys | 35 | 34 | 34 | 34 | 33 | 35 | 35 |
| NE, kcal/kg | 2,549 | 2,549 | 2,549 | 2,549 | 2,549 | 2,537 | 2,449 |
| SID Lys:NE, g/Mcal | 5.29 | 5.29 | 5.29 | 5.29 | 5.29 | 5.33 | 5.31 |
| CP, % | 20.6 | 20.3 | 20.0 | 19.6 | 19.3 | 20.9 | 20.0 |
| Ca, % | 0.65 | 0.65 | 0.65 | 0.65 | 0.64 | 0.65 | 0.61 |
| STTD P, % | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.43 |
| Ca:P | 1.10 | 1.10 | 1.11 | 1.11 | 1.12 | 1.10 | 1.08 |

[1] Phase 2 was fed from d 9 to 23 (6.8 to 12.7 kg). Phase 3 was fed from d 23 to 37 (12.7 to 20.4 kg).

[2] SPC; soy protein concentrate; PurePro Soy (Bunge, Chesterfield, MO).

[3] ESBM; enzymatically treated soybean meal; HP 300 (Hamlet Protein, Findlay, OH).

[4] Ronozyme HiPhos (DSM, Parsippany, NJ) included at 2,027 FTU/kg provided an estimated release of 0.14% STTD P.

**Table 2.4.** Ingredient composition of Exp. 2 diets (as-fed basis)[1]

| | Phase 1 | | | | | Phase 2 | | | | | Phase 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| TM-SBM replacement of SBM, %: | 0 | 25 | 50 | 75 | 100 | 0 | 12.5 | 25 | 37.5 | 50 | Common |
| Ingredient, % | | | | | | | | | | | |
| Corn | 51.27 | 51.70 | 52.09 | 52.49 | 52.88 | 57.80 | 58.02 | 58.25 | 58.48 | 58.71 | 65.56 |
| Soybean meal (47% CP) | 24.58 | 18.40 | 12.22 | 6.04 | --- | 27.24 | 23.82 | 20.40 | 16.97 | 13.55 | 30.43 |
| Thermo-mechanically processed SBM[2] | --- | 5.79 | 11.58 | 17.36 | 23.02 | --- | 3.21 | 6.41 | 9.62 | 12.82 | --- |
| Spray-dried whey | 16.65 | 16.65 | 16.65 | 16.65 | 16.65 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | --- |
| Bovine blood plasma | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | --- | --- | --- | --- | --- | --- |
| Choice white grease | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | --- |
| Calcium carbonate | 0.45 | 0.45 | 0.44 | 0.43 | 0.43 | 0.69 | 0.68 | 0.68 | 0.67 | 0.66 | 0.68 |
| Monocalcium P (21% P) | 0.93 | 0.90 | 0.89 | 0.87 | 0.85 | 0.92 | 0.92 | 0.90 | 0.89 | 0.88 | 1.00 |
| Salt | 0.40 | 0.40 | 0.41 | 0.42 | 0.43 | 0.55 | 0.56 | 0.56 | 0.57 | 0.57 | 0.60 |
| L-Lys-HCl | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| DL-Met | 0.20 | 0.21 | 0.21 | 0.22 | 0.22 | 0.22 | 0.22 | 0.23 | 0.23 | 0.23 | 0.28 |
| L-Thr | 0.18 | 0.18 | 0.18 | 0.19 | 0.19 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.25 |
| L-Trp | 0.03 | 0.03 | 0.04 | 0.04 | 0.05 | 0.05 | 0.05 | 0.06 | 0.06 | 0.06 | 0.05 |
| L-Val | 0.10 | 0.09 | 0.09 | 0.08 | 0.07 | 0.15 | 0.15 | 0.14 | 0.13 | 0.13 | 0.20 |
| L-Ile | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 0.03 |
| Zinc oxide | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | --- |
| Vitamin premix with phytase[3] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Trace mineral premix | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Choline chloride | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | --- | --- | --- | --- | --- | --- |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Calculated analysis | | | | | | | | | | | |
| SID AA, % | | | | | | | | | | | |
| Lys, % | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 | 1.25 |
| Ile:Lys | 56 | 57 | 58 | 58 | 59 | 55 | 55 | 55 | 56 | 56 | 55 |
| Leu:Lys | 117 | 117 | 118 | 118 | 119 | 112 | 112 | 113 | 113 | 113 | 111 |
| Met:Lys | 36 | 36 | 36 | 36 | 36 | 37 | 37 | 37 | 37 | 37 | 40 |
| Met and Cys:Lys | 59 | 59 | 59 | 59 | 59 | 58 | 58 | 58 | 58 | 58 | 58 |
| Thr:Lys | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 66 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Trp:Lys | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20.0 |
| Val:Lys | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 73 |
| His:Lys | 36 | 35 | 35 | 35 | 34 | 35 | 34 | 34 | 34 | 34 | 33 |
| NE, kcal/kg | 2,557 | 2,566 | 2,573 | 2,582 | 2,590 | 2,496 | 2,500 | 2,504 | 2,509 | 2,513 | 2,277 |
| SID Lys:NE, g/Mcal | 5.08 | 5.07 | 5.05 | 5.04 | 5.02 | 5.21 | 5.20 | 5.19 | 5.18 | 5.17 | 5.49 |
| CP, % | 20.1 | 19.9 | 19.7 | 19.5 | 19.2 | 19.8 | 19.7 | 19.6 | 19.5 | 19.3 | 19.8 |
| Ca, % | 0.65 | 0.64 | 0.63 | 0.62 | 0.61 | 0.70 | 0.70 | 0.69 | 0.69 | 0.68 | 0.63 |
| STTD P, % | 0.44 | 0.44 | 0.44 | 0.44 | 0.44 | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 | 0.29 |
| Ca:P | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.15 | 1.15 | 1.15 | 1.15 | 1.15 | 1.04 |

[1] Phase 1 diets were fed from d 0 to 7 (2.7 kg feed budget). Phase 2 diets were fed from d 7 to 21 (4.5 kg feed budget). Phase 3 was fed to pigs from d 21 to 28.

[2] TM-SBM; thermo-mechanically processed soybean meal; Provisoy (Cargill Animal Nutrition, Minneapolis, MN).

[3] Empirical Phytase (ADM, Chicago, IL) included at 3,132 FTU/kg provided an estimated release of 0.12% STTD P.

**Table 2.5.** Effect of soy protein concentrate (SPC) replacement of soybean meal (SBM) on growth performance and fecal dry matter (DM) of nursery pigs (Exp. 1)[1,2]

| | SPC replacement of SBM, % | | | | | ESBM, %[3] | | *P* = | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 25 | 50 | 75 | 100 | 8.50 | SEM | Linear[4] | Quadratic[4] | Protein Source[5] |
| BW, kg | | | | | | | | | | |
| d 0 | 5.6 | 5.6 | 5.6 | 5.6 | 5.6 | 5.6 | 0.02 | 0.417 | 0.568 | 0.559 |
| d 9 | 6.6 | 6.8 | 6.8 | 6.8 | 6.8 | 6.7 | 0.07 | 0.078 | 0.052 | 0.143 |
| d 23 | 12.4 | 12.8 | 12.9 | 12.8 | 12.6 | 12.7 | 0.22 | 0.546 | 0.030 | 0.529 |
| d 37 | 20.3 | 20.4 | 20.7 | 20.5 | 20.0 | 20.7 | 0.34 | 0.454 | 0.035 | 0.968 |
| Phase 1 (d 0 to 9) | | | | | | | | | | |
| ADG, g | 111 | 134 | 135 | 133 | 134 | 121 | 7.1 | 0.055 | 0.077 | 0.176 |
| ADFI, g | 141 | 149 | 143 | 161 | 154 | 140 | 8.0 | 0.158 | 0.866 | 0.842 |
| G:F, g/kg | 793 | 907 | 974 | 839 | 878 | 862 | 41.9 | 0.451 | 0.029 | 0.065 |
| Phase 2 (d 9 to 23) | | | | | | | | | | |
| ADG, g | 412 | 424 | 432 | 426 | 413 | 432 | 14.7 | 0.936 | 0.072 | 0.990 |
| ADFI, g | 533 | 560 | 559 | 572 | 568 | 551 | 16.7 | 0.024 | 0.254 | 0.620 |
| G:F, g/kg | 772 | 760 | 774 | 745 | 727 | 785 | 10.9 | 0.003 | 0.199 | 0.467 |
| Experimental period (d 0 to 23) | | | | | | | | | | |
| ADG, g | 293 | 311 | 316 | 310 | 304 | 311 | 9.5 | 0.365 | 0.030 | 0.601 |
| ADFI, g | 378 | 399 | 396 | 409 | 406 | 391 | 11.5 | 0.019 | 0.300 | 0.647 |
| G:F, g/kg | 775 | 781 | 798 | 758 | 748 | 796 | 10.1 | 0.018 | 0.024 | 0.893 |
| Common period (d 23 to 37) | | | | | | | | | | |
| ADG, g | 553 | 547 | 560 | 551 | 530 | 568 | 11.2 | 0.180 | 0.173 | 0.582 |
| ADFI, g | 815 | 814 | 831 | 818 | 788 | 825 | 25.2 | 0.270 | 0.106 | 0.765 |
| G:F, g/kg | 679 | 673 | 675 | 674 | 671 | 689 | 12.7 | 0.556 | 0.894 | 0.251 |
| Overall (d 0 to 37) | | | | | | | | | | |
| ADG, g | 389 | 400 | 408 | 401 | 389 | 407 | 8.6 | 0.960 | 0.014 | 0.881 |
| ADFI, g | 539 | 556 | 561 | 563 | 550 | 553 | 15.3 | 0.309 | 0.080 | 0.573 |
| G:F, g/kg | 722 | 721 | 729 | 712 | 706 | 736 | 8.3 | 0.087 | 0.225 | 0.506 |
| Fecal DM[6], % | | | | | | | | | | |
| d 9 | 22.7 | 23.5 | 24.8 | 25.6 | 24.5 | 21.7 | 1.36 | 0.035 | 0.177 | 0.011 |
| d 23 | 23.9 | 23.2 | 25.1 | 24.8 | 25.2 | 23.3 | 1.36 | 0.128 | 0.999 | 0.145 |

[1] A total of 360 barrows (initially 5.6 ± 0.02 kg) were used with five pigs per pen and 12 replicates per treatment.

[2] No meaningful interactions of treatment and BW block ($P > 0.10$).
[3] ESBM; enzymatically treated soybean meal; HP 300 (Hamlet Protein, Findlay, OH).
[4] Comparing the main effects of SPC replacement of SBM excluding ESBM.
[5] Comparing 8.5% SPC (50% SBM replacement) and 8.5% ESBM.
[6] No treatment × day interaction observed ($P > 0.10$).

**Table 2.6.** Effect of thermo-mechanically processed soybean meal (TM-SBM) replacement of SBM on growth performance and fecal dry matter (DM) of nursery pigs (Exp. 2)[1]

| | TM-SBM replacement of SBM, % | | | | | | *P* = | |
|---|---|---|---|---|---|---|---|---|
| Phase 1: | 0 | 25 | 50 | 75 | 100 | | | |
| Phase 2: | 0 | 12.5 | 25 | 37.5 | 50 | SEM | Linear | Quadratic |
| BW, kg | | | | | | | | |
| d 0 | 5.6 | 5.6 | 5.6 | 5.6 | 5.6 | 0.10 | 0.791 | 0.840 |
| d 7 | 6.1 | 6.3 | 6.5 | 6.4 | 6.1 | 0.13 | 0.533 | 0.001 |
| d 21 | 10.7 | 11.0 | 11.0 | 10.7 | 10.4 | 0.32 | 0.071 | 0.020 |
| d 28 | 12.9 | 13.5 | 13.2 | 13.0 | 12.6 | 0.51 | 0.165 | 0.051 |
| Phase 1 (d 0 to 7) | | | | | | | | |
| ADG, g | 57 | 88 | 103 | 100 | 65 | 9.9 | 0.205 | < 0.001 |
| ADFI, g | 197 | 205 | 208 | 201 | 182 | 8.8 | 0.062 | 0.003 |
| G:F, g/kg | 260 | 431 | 490 | 495 | 345 | 43.5 | 0.025 | < 0.001 |
| Phase 2 (d 7 to 21) | | | | | | | | |
| ADG, g | 318 | 324 | 315 | 299 | 298 | 17.9 | 0.029 | 0.528 |
| ADFI, g | 422 | 439 | 429 | 415 | 410 | 17.1 | 0.111 | 0.162 |
| G:F, g/kg | 754 | 732 | 731 | 711 | 726 | 19.8 | 0.072 | 0.288 |
| Experimental period (d 0 to 21) | | | | | | | | |
| ADG, g | 222 | 237 | 238 | 226 | 213 | 9.7 | 0.185 | 0.011 |
| ADFI, g | 339 | 353 | 348 | 337 | 327 | 10.4 | 0.069 | 0.038 |
| G:F, g/kg | 655 | 669 | 682 | 666 | 652 | 12.8 | 0.753 | 0.036 |
| Common period (d 21 to 28) | | | | | | | | |
| ADG, g | 312 | 349 | 311 | 315 | 320 | 34.3 | 0.770 | 0.736 |
| ADFI, g | 570 | 624 | 581 | 578 | 577 | 37.3 | 0.692 | 0.446 |
| G:F, g/kg | 533 | 548 | 517 | 518 | 545 | 32.0 | 0.925 | 0.422 |
| Overall (d 0 to 28) | | | | | | | | |
| ADG, g | 243 | 263 | 255 | 247 | 239 | 14.0 | 0.323 | 0.059 |
| ADFI, g | 393 | 416 | 403 | 393 | 388 | 14.5 | 0.205 | 0.089 |
| G:F, g/kg | 617 | 628 | 628 | 618 | 614 | 16.1 | 0.632 | 0.276 |
| Fecal DM[2], % | | | | | | | | |
| d 7 | 11.5 | 21.4 | 16.1 | 18.3 | 17.8 | 1.31 | 0.014 | 0.004 |
| d 21 | 19.0 | 19.5 | 18.9 | 19.0 | 19.8 | 1.31 | 0.774 | 0.786 |
| Removals, % | 7.2 | 6.8 | 4.4 | 4.4 | 2.8 | 1.63 | 0.014 | 0.759 |
| Mortality, % | 0.0 | 0.4 | 0.4 | 0.4 | 0.0 | 0.4 | 0.999 | 0.996 |
| Total removals and mortality, % | 7.2 | 7.2 | 4.8 | 4.8 | 2.8 | 1.63 | 0.014 | 0.547 |

[1] A total of 1,254 mixed-sex pigs (initially 5.6 ± 0.10 kg) were used with 18 to 20 pigs per pen and 12 pens per treatment.

[2] Treatment × day interaction (quadratic, $P = 0.024$).

# Chapter 3 - Effects of hydrolyzed yeast on weanling pig growth performance, fecal dry matter, and stress-related blood antioxidant criteria


## Abstract

A total of 360 weanling pigs (241 × 600, DNA; initially 5.4 ± 0.01 kg) were used in a 45-d study to evaluate the effects of a hydrolyzed yeast product (HY) on growth performance, fecal dry matter (DM), stress-relevant blood antioxidant criteria, and cytokine production. Pens of pigs were randomly allotted to one of six dietary treatments in a generalized randomized block design with 5 pigs per pen and 12 pens per treatment. Pigs were blocked such that there were 4 replications of light (4.3 ± 0.01 kg), medium (5.4 ± 0.01 kg), and heavy (6.5 ± 0.01 kg) weight pens per treatment. Diets were corn-soybean meal-based and consisted of a negative control (NC) diet, a positive control (PC) diet which was the NC diet + 55 mg/kg carbadox, the PC diet with 0.04% HY, and the NC diet with either 0.04, 0.08, or 0.12% HY. Treatment diets were fed in three phases from d 0 to 10 (phase 1), d 10 to 24 (phase 2), and d 24 to 45 (phase 3). On d 10 and 24, fecal samples were collected from the same three initially randomly selected pigs in each pen to determine fecal DM and fecal scores. Blood samples were collected on d 10 and 45 from the same representative pig in each pen for total antioxidant capacity (TAC), superoxide dismutase (SOD), and serum cytokine production. Overall (d 0 to 45), final BW increased ($P$ < 0.05) while ADG and ADFI tended to increase ($P$ < 0.10) for pigs fed diets containing carbadox compared to pigs fed diets without carbadox. Feed efficiency improved ($P$ = 0.017) when 0.04% HY was added to the negative and positive control diets. On d 10, increasing HY in diets without carbadox increased fecal DM (linear, $P$ = 0.005). Additionally, pigs fed carbadox had increased

($P < 0.05$) fecal DM on d 10 and 24 compared to pigs not fed carbadox. There were no significant treatment differences observed on d 10 for TAC; however, TAC decreased (linear, $P = 0.008$) on d 45 as HY increased in the diet. Increasing HY tended to decrease (linear, $P = 0.095$) serum SOD activity on d 10 while no significant treatment differences were observed on d 45. In summary, pigs fed carbadox had increased overall ADG and fecal DM. Increasing HY inclusion did not affect growth performance; however fecal DM was improved during the early nursery period. Additionally, feeding 0.04% HY improved overall G:F.

**Key Words:** cytokine production, fecal dry matter, hydrolyzed yeast, nursery pig, superoxide dismutase, total antioxidant capacity

## Introduction

Weaned pigs often experience reduced growth performance and increased prevalence of diarrhea due to the transition from a milk-based diet to a dry, cereal-based diet (Kluess et al., 2009; Campbell et al., 2013). At weaning, beneficial *Lactobacillus* populations are reduced, which compromises gut barrier integrity and allows pathogenic bacteria, like *Escherichia coli,* to proliferate (Fairbrother et al., 2007). The proliferation of the *E. coli* pathotype, enterotoxigenic *E. coli* (ETEC), is a significant contributor to postweaning diarrhea which can adversely affect intestinal integrity and growth performance (Yan et al., 2023). To help overcome these challenges and maintain weanling pig health, in-feed antibiotics have been widely utilized to maintain gut microbiota and improve growth performance.

Carbadox, a quinoxaline class of antimicrobials, is used to control and prevent enteric health challenges and improve growth performance in pigs (Dasenaki et al., 2023). It inhibits bacterial growth by intercalating into DNA, leading to mutations that hinder replication (Chen et al., 2009). At low doses, carbadox can be used to improve feed efficiency (Dritz et al., 2002). Due to concerns of antibiotic resistance, the U.S. Food and Drug Administration prohibited the use of certain in-feed antibiotics important for human medicine as growth promoters in livestock in 2017 (FDA, 2013). This ban on antibiotic growth promotors (AGP) has driven interest in finding alternatives with similar health benefits.

Nutritional strategies that may act as a substitute for AGP such as probiotics, prebiotics, phytogenic additives, and organic acids have been extensively explored. Yeast-based feed additives, specifically *Saccharomyces cerevisiae*, have been of interest due to their potential to improve gut health, nutrient digestibility, and growth performance (Schiavone et al., 2015; Sampath et al., 2023). There are different kinds of yeast products such as yeast extracts, yeast cultures, yeast cell walls, yeast cell wall components, and live yeast cells. Previous literature has shown improvements in intestinal morphology, diarrhea prevalence, and intestinal inflammation when utilizing dietary yeast products during the nursery phase (Shen et al., 2009; Zanello et al., 2011; Trckova et al., 2014). However, studies measuring growth performance have been variable. Van Der Peet-Schwering et al. (2007) and Shen et al. (2009) observed improvements in growth performance when yeast culture was utilized in the diet, while others using live yeast have shown no benefit (van Heugten et al., 2003; Chance et al., 2021). These inconsistent findings could stem from variations in the types of yeast products used, the inclusion and duration of supplementation, diet composition, and health status of the pigs.

A novel hydrolyzed yeast product (HY; Ceretide S, Boon Lay Way, Singapore) is derived from cultivated *S. cerevisiae* yeast cells, which undergo enzymatic hydrolysis using exogenous enzymes. This process results in a product that contains the whole-cell components of yeast resulting in a composition that also contains metabolic by-products such as enzymes, oligopeptides, vitamins, saccharides, organic acids, and other fermentation metabolites. Hydrolyzed yeast is thought to combine the beneficial aspects of yeast extract (soluble nutrients) and yeast cell walls (mannan-oligosaccharides and β-glucans).

Given the wide variety of yeast products available, it is important to evaluate the efficacy of new products. Therefore, the objective of this study was to evaluate the effects of HY with or without carbadox on growth performance, fecal DM, stress-related blood antioxidant criteria, and serum cytokine production in nursery pigs. It was hypothesized that the addition of HY would improve nursery pig growth performance and fecal DM.

## Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in this experiment (IACUC #4942).

This study was conducted at the Kansas State University Swine Research and Teaching Center in Manhattan, KS. A total of 360 weanling pigs (241 × 600 DNA; initially 5.4 ± 0.01 kg) were used in a 45-d growth trial. Pigs were weaned at approximately 18 d of age and blocked by initial body weight (BW). Pens of pigs were randomly allotted to one of six dietary treatments in a generalized randomized block design. There were 5 pigs per pen, and within each block, there were 4 pens per weight group [light (4.3 ± 0.01 kg), medium (5.4 ± 0.01 kg), and heavy (6.5 ± 0.01 kg)] for a total of 12 replications per treatment. Diets were corn-soybean meal-based and consisted of a negative control (NC) diet, a positive control (PC) diet which was the NC diet + 55 mg/kg carbadox (Mecadox 2.5, Phibro; Teaneck, NJ), the PC diet with 0.04% hydrolyzed yeast (HY), and the NC diet with either 0.04, 0.08, or 0.12% HY (Table 1). All diets were formulated to 1.35% standardized ileal digestible (SID) Lys in phase 1 and 2 and 1.30% SID Lys in phase 3. All diets met or exceeded the nutrient requirement estimates of other amino acids as a ratio to Lys (NRC, 2012). Pigs were fed treatment diets in three phases from d 0 to 10 (phase 1), d 10 to 24 (phase 2), and d 24 to 45 (phase 3). Treatment diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS and fed in meal form for all phases. Each pen contained a 3-hole, dry self-feeder and a nipple waterer for *ad libitum* access to feed and water.

Pig weights and feed disappearance were measured on d 0, 10, 24, and 45 to determine average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F). On d 10 and 24, fecal samples were collected from the same 3 initially randomly selected pigs in each pen to determine fecal dry matter (DM) and fecal score. Fecal samples were dried at 55°C in a forced air oven for 48 h, and the ratio of dried to wet fecal weight determined the percentage fecal DM. The average of the 3 samples from each pen was then used for statistical analysis. Fecal samples were also scored in the bag by three observers on d 10 and 24 using a 0 to 4 scoring system: 0 = hard, pellet-like lumps; 1 = firm, formed feces; 2 = normal feces; 3 = mild looseness; and 4 = diarrhea.

Blood samples were collected on d 10 and 45 from the same median-weight pig in each pen for total antioxidant capacity (TAC), superoxide dismutase (SOD), and serum cytokine production. Blood samples were collected with blood collection tubes (Covidien Monoject blood collection tubes, silicone-coated tubes with red stoppers, no additive, 10 mL; Medtronic, Minneapolis, MN). The clotted samples were centrifuged at 1,500 × g for 30 min, after which the resulting serum supernatants were divided into 4 polypropylene tubes (PR1MA microcentrifuge tubes, natural boil-proof; Midwest Scientific, St. Louis, MO) and stored as aliquots at -80°C until use. Serum cytokine panel analyses (GM-CSF, IFNγ, IL-1α, IL-1ra, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, and TNFα) was conducted at Eve Technologies (Calgary, AB, Canada). Serum TAC and SOD analyses were conducted at the Kansas State University Swine Nutrition Laboratory (Manhattan, KS). Both serum SOD and TAC samples were analyzed in duplicate in 96-well microplates with an intra-assay CV ≤ 5.0%. Assay kits for SOD and TAC were from Cayman Chemical Company (Ann Arbor, MI; # 706002) and Cell Biolabs Inc. (San Diego, CA; # STA-360), respectively.

### Statistical Analysis

Growth performance, fecal DM, and cytokine panel data were analyzed as a generalized randomized block design as a one-way ANOVA using the lmer function from the lme4 package in R studio (Version 4.3.1, R Core Team. Vienna, Austria) with pen serving as the experimental unit and dietary treatment and weight block as fixed effects. Linear and quadratic contrasts were tested within increasing levels of HY in diets without carbadox. Additionally, the main effect of HY was evaluated plus the interaction of HY and carbadox. The main effect of carbadox was evaluated by comparing the average of 0 and 0.04% HY in the presence and absence of carbadox. Additionally, a comparison was made between carbadox with 0.04% HY and carbadox without HY. Fecal DM and serum samples were analyzed using the fixed effects of day, treatment, block, and the associated interactions accounting for repeated measures over time.

For serum cytokine data, the data were analyzed with the raw fluorescence intensity value based on Breen et al. (2015) with a log10 transformation for statistical analysis, which was transformed back for reporting of treatment means. For serum TAC and SOD assays, data were analyzed using the GLIMMIX procedure of SAS OnDemand for Academics (SAS Institute Inc., Cary, NC) with microtiter plate used in the model as a random intercept. Fecal scores were summarized using the FREQ procedure of SAS OnDemand for Academics and reported as a percentage of observations within each score category by treatment. Fecal scores are reported using descriptive statistics due to a lack of model fit. When treatment was a significant source of variation, differences were determined by pairwise comparison using the Tukey-Kramer multiplicity adjustment to control for Type I Error. Results were considered significant with $P \leq 0.05$ and marginally significant with $0.05 < P \leq 0.10$.

## Results

There were no significant BW block interactions for growth performance, fecal DM, or serum antioxidant data, therefore, interpretation is based on the main effect of dietary treatment.

### Growth Performance

From d 0 to 10 (phase 1), there was a main effect of carbadox, where pigs fed diets containing carbadox had increased ($P \leq 0.025$) ADG and d 10 BW compared to those not fed carbadox with either none or 0.04% HY (Table 2). From d 10 to 24 (phase 2), an increase in G:F was observed ($P = 0.041$) when 0.04% HY was added, regardless of carbadox inclusion. Also, d 24 BW was greater ($P = 0.048$) in pigs fed diets containing carbadox compared to pigs fed diets without carbadox. From d 24 to 45 (phase 3), ADFI increased ($P = 0.041$) for pigs fed diets containing carbadox compared to pigs not fed carbadox, which led to a tendency ($P = 0.087$) for increased ADG due to carbadox. Also, pigs fed 0.04% HY and carbadox tended to increase ($P < 0.10$) G:F compared to pigs fed carbadox without HY.

Overall (d 0 to 45), final BW increased ($P = 0.026$) and ADG and ADFI tended to increase ($P \leq 0.089$) for pigs fed diets containing carbadox compared to pigs fed diets without carbadox with either none or 0.04% HY. Gain-to-feed ratio increased ($P = 0.017$) when 0.04%

HY was added, regardless of carbadox inclusion. The combination of 0.04% HY and carbadox increased ($P < 0.05$) G:F compared to pigs fed carbadox alone.

### Fecal DM and Scoring

On d 10, increasing HY in diets without carbadox increased (linear, $P = 0.005$) fecal DM (Table 3). Additionally, pigs fed carbadox had increased ($P \leq 0.009$) fecal DM on d 10 and 24 compared to pigs not fed carbadox. Fecal scores are reported using descriptive statistics due to a lack of model fit. For fecal scoring on d 10, the pigs fed the negative control had a higher numerical incidence of diarrhea than pigs fed the other dietary treatments (Figure 1). The frequency of diarrhea (score of 4) decreased as HY increased in pigs fed diets either with or without carbadox. On d 24, pigs fed 0.04% HY numerically had the highest frequency of diarrhea with the frequency decreasing as the level of HY increased. Pigs fed the diet with carbadox alone had the lowest frequency of diarrhea.

### Serum Cytokines

On d 10, pigs fed 0.04% HY in diets containing carbadox tended to have decreased ($P < 0.10$) cytokines IFNγ, IL-1α, IL-2, IL-4, IL-6, IL-10, and IL-18 compared to pigs fed carbadox alone (Table 3). Cytokines GM-CSF, IL-2, IL-4, and TNFα decreased then increased (quadratic, $P < 0.05$) on d 10 and IL-1α, IL-1β, IL-6, and IL-10 tended to decrease then increase (quadratic, $P < 0.10$) as HY increased in diets without carbadox with the lowest concentration observed at an inclusion level of 0.08% HY or 0.04% for GM-CSF. IFNγ increased (linear, $P = 0.015$) as HY increased on d 10 although a tendency for a quadratic response (quadratic, $P = 0.065$) was also observed. Cytokines IL-8 and IL-10 tended to decrease ($P \leq 0.097$) when 0.04% HY was added to the diet, regardless of carbadox inclusion. On d 45, no significant treatment differences were observed.

### Antioxidant Status

No significant treatment differences were observed on d 10 for TAC. Total antioxidant capacity decreased (linear, $P = 0.008$) on d 45 as HY increased. Increasing HY tended to decrease (linear, $P = 0.095$) serum SOD activity on d 10. Also, there was a tendency for an increase ($P < 0.10$) in serum SOD activity in pigs fed 0.04% HY with carbadox compared to pigs fed carbadox without HY. No significant treatment differences were observed on d 45.

## Discussion

*Saccharomyces cerevisiae* yeast products are available in various forms, including live yeast (probiotic), yeast cultures (prebiotic), yeast extracts (postbiotic), yeast cell walls, and hydrolyzed yeast. Research on yeast-based products primarily focus on nursery pigs, as they are highly susceptible to gut pathogens and diarrhea due to dietary transitions, immature gut health, and social stressors (Chance et al., 2021; Long et al., 2021). Studies have reported benefits of yeast supplementation on immune function and gut morphology (Shen et al., 2009; Namted et al., 2022). Additionally, yeast products contain nucleotides, free amino acids, and vitamins (Demirgul et al., 2022), which can support intestinal development and may contribute to improved nutrient digestibility. However, these positive effects do not always translate into improved growth performance, likely due to the variability in yeast composition due to differences in manufacturing processes, culture conditions, and preparation techniques (Aguilar-Uscanga and François, 2003).

Some studies have demonstrated positive effects of added yeast products on weanling pig growth performance. For instance, Boontiam et al. (2022) observed a linear increase in BW and ADG with increasing hydrolyzed yeast (0, 5, and 10% of the diet). These improvements can be attributed to enhanced crude protein digestibility, and improved intestinal morphology

(Boontiam et al., 2022). However, the hydrolyzed yeast used by Boontiam et al. (2022) is a different product than what was tested in the present study, as well as at a higher inclusion rate. Similarly, Shi and Kim (2019) reported improvements in growth performance with added yeast extract complex (0.1 and 0.2%) from a mixture of *Kluyveromyces maxianus* and *S. cerevisiae* containing β-glucan, mannan, nucleotide protease, and α-amylase, noting increased overall ADG and a tendency for increased ADFI, along with a tendency for increased G:F when pigs were approximately 25 kg. In contrast, the present study observed no differences in ADG or ADFI, despite containing similar components of β-glucan, mannan, and enzymes, potentially due to the considerably lower inclusion rates of HY (0.04, 0.08, and 0.12%). Molist et al. (2014) also did not observe differences in growth performance when supplementing 0.2% of a different hydrolyzed *S. cerevisiae* yeast derivative. However, they observed a tendency for increased G:F, which is comparable to the increased G:F in the present study with 0.04% added HY. Similarly, Liu et al. (2017) observed increased G:F when supplementing *S. cerevisiae* cell wall extract at 0.5 and 1.0% of the diet. These findings illustrate the potential of *S. cerevisiae*-derived products to improve G:F, although responses may vary depending on the specific form and inclusion level.

Carbadox has been widely used in swine production due to its ability to regulate gut microbiota (Looft et al., 2014), promote growth and enhance feed efficiency (Yen et al., 1985), as well as its capacity to reduce inflammation (He et al., 2020). By suppressing the proliferation of harmful bacteria, carbadox can improve digestion and nutrient absorption by reducing microbial competition, ultimately improving growth performance (Gaskins et al., 2002). Research on the effects of carbadox dating back to the late 1960s supports its ability to improve growth performance in nursery pigs (Thrasher et al., 1969; Roof and Mahan, 1982; Nabuurs et al., 1990). The findings from the present study demonstrate that added carbadox improved overall ADG, ADFI, and BW gain compared to pigs fed diets without carbadox. Notably, the increase in BW gain occurred without a proportional improvement in G:F, suggesting that the enhanced growth performance was primarily driven by greater feed intake. This observation aligns with Yen and Pond (1993), who observed that pigs fed carbadox at 55 mg/kg exhibited greater BW and a tendency for an increased feed intake without significant changes in G:F. Furthermore, carbadox supplementation (55 mg/kg) has been shown to effectively improve the growth performance of weanling pigs (He et al., 2020), further supporting the results observed in the current study.

Oxidative stress in weanling pigs can result from various factors like environmental stressors, disease, and dysbiosis in the gut microbiome. Total antioxidant capacity (TAC) measures the overall antioxidant potential in a biological sample, while superoxide dismutase (SOD) specifically quantifies the activity of an enzyme crucial to the antioxidant defense system (Bafana et al., 2010). While there are no established reference values for optimal TAC and SOD levels, higher concentrations generally indicate improved antioxidant status. Previous research has shown that including yeast-based products like cell wall extract at either 0.05, 0.10, or 0.15% (Liu et al., 2017) and live yeast at 0.2% (Long et al., 2021) in nursery pig diets can enhance SOD activity, suggesting an increase in antioxidative capacity. In contrast, the present study observed no differences in SOD activity or TAC levels between pigs fed the control diet and 0.04% added HY. However, an unexpected decrease in both TAC and SOD were observed with increasing HY, indicating a reduction in antioxidant capacity. A possible explanation is that higher inclusions of HY may have been potentially acting as a physiological stressor rather than providing a beneficial effect on oxidative status, as indicated by the elevated cytokines observed with this diet.

Cytokines are signaling proteins that mediate communication between immune cells and regulate inflammation, tissue repair, and immune responses. A decrease in pro-inflammatory cytokine levels (IFNγ, IL-1β, IL-2, IL-6, IL-8, IL-12, IL-18, and TNFα) indicates improved inflammation status, suggesting that pigs may allocate less energy and AA toward immune overexpression, potentially enhancing overall energy and AA utilization (Dinarello, 2000). Anti-inflammatory cytokines such as Il-4 and IL-10 reduce inflammation and regulate the immune response by reducing the pro-inflammatory cytokine response. Christensen et al. (2022) observed no differences in cytokines IL-6, IL-10, or TNFα in pigs fed diets with enzymatically treated yeast compared with pigs fed the control diet. Fu et al. (2019) observed no differences in cytokines IL-1β or TNFα, but an increase in IL-10 in pigs fed a diet with yeast hydrolysates compared with those fed a control diet. Interleukins (IL) are a group of cytokines that act as chemical signals between white blood cells while interferons (IFN) help resist viral infections and regulate innate and adaptive immune responses. Zanello et al. (2011) observed that yeast culture reduced the intestinal inflammation caused by pathogens through the inhibition of pro-inflammatory cytokine expression (TNFα, IL-1α, IL-6, and IL-8) induced by ETEC. In the present study, 0.04% HY inclusion with carbadox tended to decrease IFNγ, IL-1α, IL-2, IL-4, IL-6, IL-10, and IL-18 compared to feeding carbadox without HY supplementation, suggesting that these pigs had less inflammation or immune activation, which indicates an additive effect of the two products. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is produced by activated T lymphocytes and other immune cells to control the differentiation and functional activation of hematopoietic cells following infection. Cytokines GM-CSF, IL-2, IL-4, and TNFα decreased then increased on d 10 and IL-1α, IL-1β, IL-6, and IL-10 tended to decrease then increase with increasing HY in diets without carbadox, again suggesting that higher inclusions of HY may have caused a physiological stressor as the cytokine levels were high. The observed increase in cytokines levels may be attributed to the yeast components, specifically β-glucans, which are recognized by receptors like dectin-1 on immune cells such as macrophages and neutrophils (Goodridge et al., 2009). The binding of β-glucans can trigger the production of pro-inflammatory cytokines such as TNF-α and IL-1β (Fu et al., 2023). In the present study, carbadox had no effect on cytokine concentrations. However, previous literature has shown that carbadox can reduce pro-inflammatory cytokines like TNFα (He et al., 2020) and Il-1β (Hung et al., 2020).

Complex carbohydrates, such as β-glucans and mannan-oligosaccharides (MOS), impact the gut microbiota of the pig by binding pathogenic bacteria like *E. coli* and Salmonella, decreasing their ability to adhere to intestinal walls (Moreira dos Anjos et al., 2019). These components are found in products like hydrolyzed yeast that contain components of the yeast cell wall. Lee et al. (2021) observed that an inclusion of 0.05% yeast cell wall tended to decrease the frequency of diarrhea compared to the control diet during the first two weeks post-weaning. A similar finding was observed in the present study, where pigs fed diets containing HY had higher fecal DM (indicating lower diarrhea) than those fed the control diet. Due to their ability to bind pathogenic bacteria, yeast-products containing β-glucans and MOS have been widely studied for their effects in challenge models. Trckova et al. (2014) found that pigs fed live yeast *S. cerevisiae* at 0.5% had a lower daily diarrhea score, indicating less diarrhea, as well as a shorter duration of diarrhea compared to the control group when the pigs are orally challenged with ETEC K88. Although pigs were not inoculated with *E. coli* in the present study, *E. coli* can still be present under non-challenge conditions. Through diagnostic testing of a representative fecal sample for this group of pigs, F18 *E. coli* was identified via polymerase chain reaction analysis with genes for heat stable toxin 1a (st1a), heat stable toxin 1b (st1b), and heat labile toxin (lt) along with rotavirus group A. A similar response in a non-challenge setting was observed by Boontiam et al. (2020) who noted lower diarrhea rate with increasing hydrolyzed yeast of a different yeast product, aligning with the findings from the present study where there was an increase in fecal DM as HY inclusion increased.

Additionally, pigs fed diets containing carbadox had a higher fecal DM. Carbadox inhibits the growth of enteric pathogens and alters the gut microbial composition, which can lead to decreased prevenance of diarrhea. Because of this, more research has been done evaluating its effect in pathogen challenges. Kim et al. (2021) observed that ETEC F18 challenged pigs fed diets containing carbadox had lower average diarrhea scores and frequency of diarrhea, like observed in pigs fed carbadox in the present study. In the present study, there was a correlation between diarrhea occurrence, as measured by fecal scoring, and fecal DM. As HY inclusion increased, the frequency of diarrhea decreased and fecal DM increased, indicating firmer stools.

In conclusion, 0.04% HY improved G:F and increasing HY improved fecal DM in the early nursery phase. However, increasing HY inclusion did not result in improved growth performance. Pigs fed carbadox had increased final BW and firmer stools compared with those fed no carbadox and the same HY inclusion. Addition of 0.04% HY with carbadox tended to reduce serum cytokine concentrations (IFN$\gamma$, IL-1$\alpha$, IL-2, IL-4, IL-6, IL-10, and IL-18) compared to carbadox alone. Additionally, serum cytokine concentrations (TNF$\alpha$, IL-1$\alpha$, IL-1$\beta$, IL-2, IL-4, IL-6, and IL-10) tended to decrease then increase as HY increased with the lowest concentration observed at an inclusion level of 0.08%.

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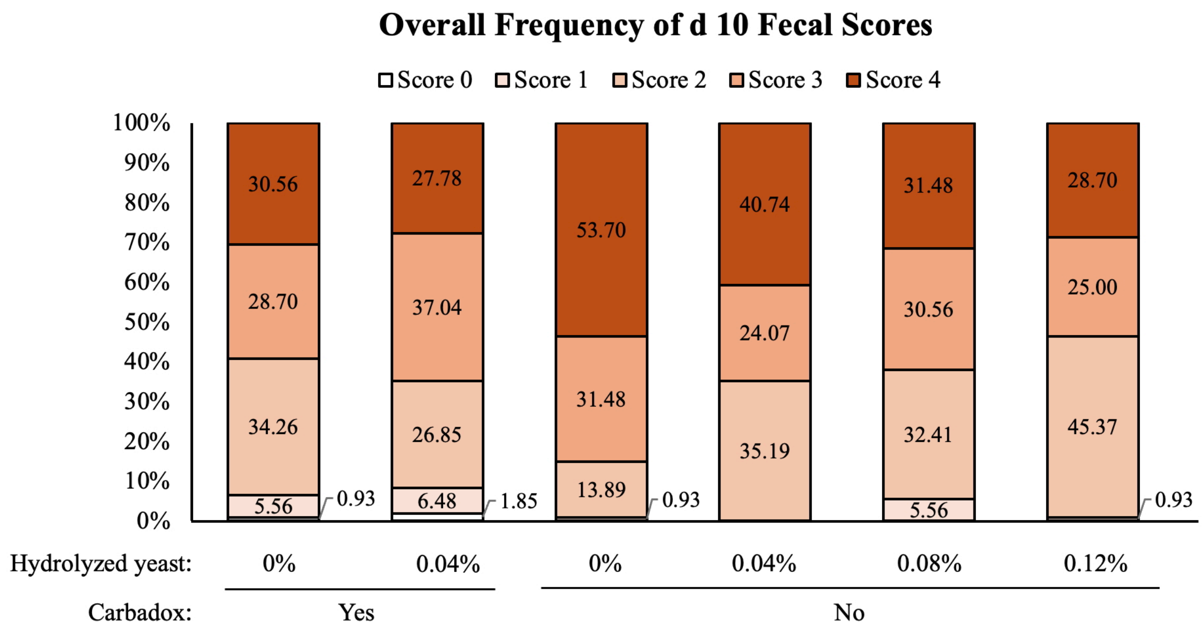


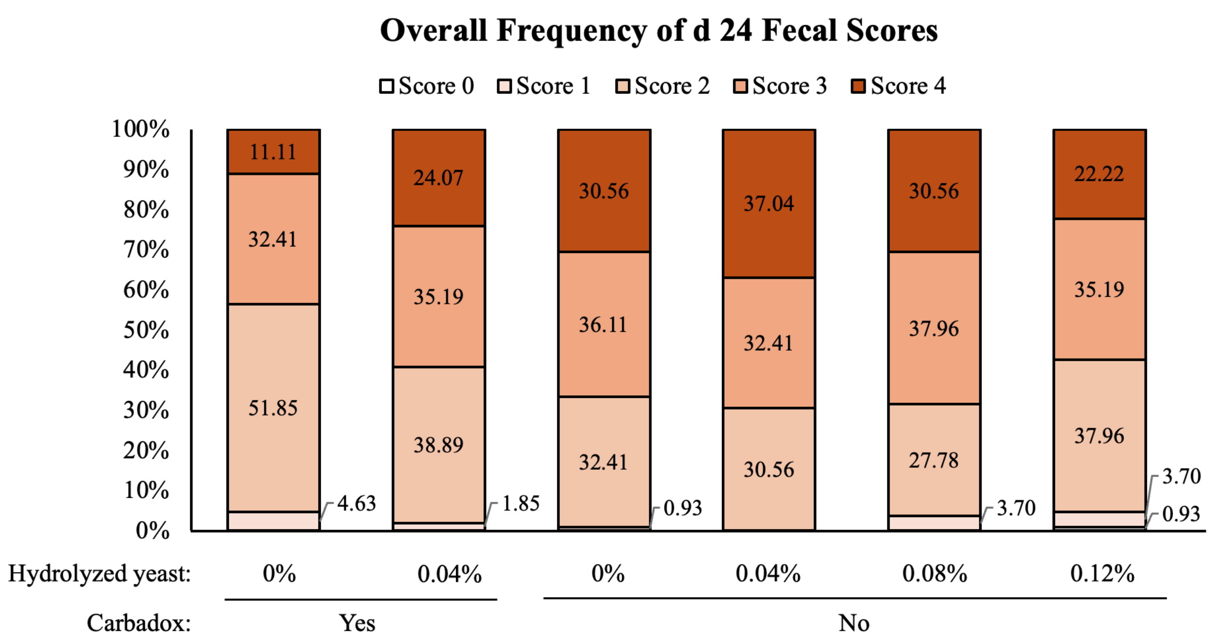




**Figure 3.1.** Effect of hydrolyzed yeast on fecal score frequency. Fecal scores are presented on a 4-point scale: 0 = hard, pellet-like lumps; 1 = firm, formed feces; 2 = normal feces; 3 = mild looseness; and 4 = diarrhea by three observers on d 10 and 24. Fecal scores are reported using descriptive statistics due to a lack of model fit.

**Table 3.1.** Composition of experimental diets (as-fed basis)[1]

| | | Phase 1 | | Phase 2 | | Phase 3 | |
|---|---|---|---|---|---|---|---|
| Ingredient, % | Carbadox: | Yes | No | Yes | No | Yes | No |
| Corn | | +/- | +/- | +/- | +/- | +/- | +/- |
| Soybean meal (47% CP) | | 16.13 | 16.07 | 23.92 | 23.86 | 29.81 | 29.75 |
| Enzymatically treated SBM[2] | | 5.00 | 5.00 | 4.50 | 4.50 | --- | --- |
| Whey powder | | 20.00 | 20.00 | 10.00 | 10.00 | --- | --- |
| Fish meal | | 4.50 | 4.50 | --- | --- | --- | --- |
| Lactose | | 4.50 | 4.50 | --- | --- | --- | --- |
| Soybean oil | | 1.00 | 1.00 | --- | --- | --- | --- |
| Calcium carbonate | | 0.20 | 0.37 | 0.53 | 0.70 | 0.68 | 0.85 |
| Monocalcium P (21% P) | | 0.45 | 0.45 | 1.00 | 1.00 | 1.05 | 1.05 |
| Salt | | 0.33 | 0.33 | 0.55 | 0.55 | 0.60 | 0.60 |
| L-Lys-HCl | | 0.43 | 0.43 | 0.50 | 0.50 | 0.50 | 0.50 |
| DL-Met | | 0.22 | 0.22 | 0.23 | 0.23 | 0.20 | 0.20 |
| L-Thr | | 0.21 | 0.21 | 0.23 | 0.23 | 0.23 | 0.23 |
| L-Trp | | 0.04 | 0.04 | 0.03 | 0.03 | 0.03 | 0.03 |
| L-Val | | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 |
| Trace mineral premix | | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Vitamin premix | | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Phytase[3] | | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| Carbadox[4] | | 1.00 | --- | 1.00 | --- | 1.00 | --- |
| Hydrolyzed yeast[5] | | +/- | +/- | +/- | +/- | +/- | +/- |
| Total | | 100 | 100 | 100 | 100 | 100 | 100 |
| Calculated analysis | | | | | | | |
| SID Lys, % | | 1.35 | 1.35 | 1.35 | 1.35 | 1.30 | 1.30 |
| NE, kcal/kg | | 2,555 | 2,576 | 2,434 | 2,455 | 2,407 | 2,428 |
| CP, % | | 20.2 | 20.2 | 20.7 | 20.7 | 20.5 | 20.5 |
| Ca, % | | 0.71 | 0.71 | 0.71 | 0.71 | 0.72 | 0.72 |
| STTD P, % | | 0.44 | 0.44 | 0.41 | 0.41 | 0.36 | 0.36 |
| Ca:P | | 1.11 | 1.11 | 1.11 | 1.11 | 1.18 | 1.18 |

[1] Phase 1 diets were fed from d 0 to 10 (5.4 to 6.2 kg). Phase 2 diets were fed from d 10 to 24 (6.2 to 11.6 kg). Phase 3 diets were fed from d 24 to 45 (11.6 to 26.8 kg).

[2] ESBM; enzymatically treated soybean meal; HP 300 (Hamlet Protein, Findlay, OH).

[3] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) added at 811 FTU/kg provided an estimated release of 0.14% STTD P.

[4] Mecadox 2.5 (Phibro; Teaneck, NJ) included at 55 mg/kg of complete feed.

[5] Ceretide S (Planet Bioscience; Boon Lay Way, Singapore) added at the expense of corn.

**Table 3.2.** Effects of hydrolyzed yeast (HY) and carbadox on growth performance and fecal dry matter (DM) of nursery pigs[1]

| Carbadox[3]: | Yes | | No | | | | | $P^2 =$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | HY in No Carbadox[4] | | | |
| HY, %: | 0.00 | 0.04 | 0.00 | 0.04 | 0.08 | 0.12 | SEM | Linear | Quadratic | 0.04% HY[5] | Carbadox[6] |
| BW, kg | | | | | | | | | | | |
| d 0 | 5.4 | 5.4 | 5.4 | 5.4 | 5.4 | 5.4 | 0.01 | 0.224 | 0.379 | 0.282 | 0.232 |
| d 10 | 6.3 | 6.4 | 6.1 | 6.0 | 6.1 | 6.0 | 0.11 | 0.878 | 0.880 | 0.905 | 0.018 |
| d 24 | 11.9 | 12.3 | 11.5 | 11.4 | 11.2 | 11.4 | 0.32 | 0.657 | 0.616 | 0.749 | 0.048 |
| d 45 | 27.2 | 28.2 | 26.5 | 26.5 | 26.0 | 26.1 | 0.50 | 0.388 | 0.909 | 0.375 | 0.026 |
| Phase 1 (d 0 to 10) | | | | | | | | | | | |
| ADG, g | 92 | 96 | 71 | 67 | 71 | 65 | 11.4 | 0.773 | 0.958 | 0.999 | 0.025 |
| ADFI, g | 132 | 121 | 122 | 113 | 112 | 105 | 9.8 | 0.234 | 0.901 | 0.295 | 0.353 |
| G:F, g/kg | 676 | 687 | 573 | 552 | 503 | 582 | 99.6 | 0.959 | 0.604 | 0.960 | 0.223 |
| Phase 2 (d 10 to 24) | | | | | | | | | | | |
| ADG, g | 399 | 408 | 375 | 380 | 350 | 371 | 18.3 | 0.618 | 0.652 | 0.697 | 0.141 |
| ADFI, g | 543 | 546 | 522 | 507 | 494 | 511 | 24.0 | 0.660 | 0.492 | 0.790 | 0.204 |
| G:F, g/kg | 732 | 750 | 716 | 754 | 710 | 724 | 14.2 | 0.772 | 0.389 | 0.041 | 0.654 |
| Phase 3 (d 24 to 45) | | | | | | | | | | | |
| ADG, g | 728 | 751 | 714 | 721 | 703 | 699 | 12.9 | 0.269 | 0.686 | 0.248 | 0.087 |
| ADFI, g | 1,072 | 1,073 | 1,022 | 1,028 | 1,012 | 1,004 | 23.6 | 0.502 | 0.734 | 0.877 | 0.041 |
| G:F[7], g/kg | 680 | 702 | 699 | 705 | 698 | 699 | 8.9 | 0.850 | 0.827 | 0.122 | 0.213 |
| Overall (d 0 to 45) | | | | | | | | | | | |
| ADG, g | 482 | 490 | 458 | 469 | 444 | 451 | 12.0 | 0.359 | 0.854 | 0.430 | 0.058 |
| ADFI, g | 696 | 685 | 656 | 663 | 639 | 643 | 18.7 | 0.435 | 0.932 | 0.906 | 0.089 |
| G:F[8], g/kg | 694 | 717 | 699 | 711 | 697 | 703 | 7.7 | 0.937 | 0.645 | 0.017 | 0.943 |
| Fecal DM[9], % | | | | | | | | | | | |
| d 10 | 17.6 | 17.3 | 12.3 | 15.0 | 16.3 | 17.3 | 1.29 | 0.005 | 0.493 | 0.335 | 0.003 |
| d 24 | 20.6 | 18.8 | 16.2 | 16.7 | 18.3 | 18.2 | 1.29 | 0.177 | 0.831 | 0.578 | 0.009 |

[1] A total of 360 pigs (initially 5.4 ± 0.01 kg) were used in a 45-d growth study with 5 pigs per pen and 12 replicates per treatment.
[2] No significant interactive responses ($P > 0.10$) of carbadox and HY were observed.
[3] Mecadox 2.5 (Phibro; Teaneck, NJ) included at 55 mg/kg of complete feed.
[4] Increasing HY with no carbadox.
[5] Comparing the mean of 0% HY with and without carbadox vs. the mean of 0.04% HY with and without carbadox.

[6] Comparing the mean of 0% and 0.04% HY with carbadox vs. the mean of 0 and 0.04% HY without carbadox.

[7,8] Pairwise comparison of carbadox with 0.04% HY vs. carbadox without HY, [7]$P < 0.10$. [8]$P < 0.05$.

[9] No significant treatment × day interactions ($P > 0.10$).

**Table 3.3.** Effects of hydrolyzed yeast (HY) and carbadox on serum cytokine profile (fluorescence intensity values) and stress-relevant blood antioxidant criteria (SOD and TAC)[1,2,3]

| Carbadox[4]: | Yes | | No | | | | | $P^5$ = | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | HY in No Carbadox[6] | | | |
| HY, %: | 0.00 | 0.04 | 0.00 | 0.04 | 0.08 | 0.12 | SEM | Linear | Quadratic | 0.04% HY[7] | Carbadox[8] |
| GM-CSF | | | | | | | | | | | |
| d 10 | 32.2 | 17.2 | 20.4 | 17.4 | 17.6 | 105.4 | 1.40 | 0.001 | 0.003 | 0.231 | 0.491 |
| d 45 | 9.5 | 9.1 | 8.1 | 8.4 | 9.8 | 11.4 | 1.40 | 0.428 | 0.846 | 0.981 | 0.707 |
| IFNγ | | | | | | | | | | | |
| d 10[9] | 229.5 | 107.2 | 103.6 | 98.1 | 106.0 | 330.0 | 1.39 | 0.015 | 0.065 | 0.205 | 0.170 |
| d 45 | 21.0 | 20.5 | 19.9 | 29.0 | 27.9 | 36.2 | 1.39 | 0.226 | 0.862 | 0.582 | 0.647 |
| IL-1α | | | | | | | | | | | |
| d 10[9] | 262.4 | 87.8 | 241.3 | 196.1 | 88.7 | 321.7 | 1.51 | 0.970 | 0.064 | 0.107 | 0.371 |
| d 45 | 72.3 | 44.5 | 57.5 | 75.1 | 43.3 | 91.8 | 1.51 | 0.637 | 0.546 | 0.786 | 0.714 |
| IL-1β | | | | | | | | | | | |
| d 10 | 262.7 | 113.6 | 236.4 | 193.8 | 105.2 | 338.3 | 1.47 | 0.784 | 0.071 | 0.170 | 0.570 |
| d 45 | 62.5 | 35.6 | 36.8 | 45.2 | 28.9 | 59.2 | 1.47 | 0.563 | 0.496 | 0.636 | 0.697 |
| IL-1ra | | | | | | | | | | | |
| d 10 | 541.3 | 331.2 | 480.1 | 401.7 | 348.2 | 629.4 | 1.32 | 0.586 | 0.159 | 0.220 | 0.894 |
| d 45 | 83.0 | 66.8 | 76.3 | 81.5 | 60.6 | 113.6 | 1.32 | 0.466 | 0.303 | 0.783 | 0.833 |
| IL-2 | | | | | | | | | | | |
| d 10[9] | 246.7 | 82.4 | 233.1 | 168.2 | 72.3 | 292.2 | 1.56 | 0.932 | 0.049 | 0.103 | 0.450 |
| d 45 | 50.9 | 32.8 | 41.6 | 57.3 | 32.7 | 66.5 | 1.56 | 0.666 | 0.651 | 0.888 | 0.681 |
| IL-4 | | | | | | | | | | | |
| d 10[9] | 268.6 | 102.3 | 249.0 | 191.3 | 90.3 | 356.7 | 1.51 | 0.855 | 0.041 | 0.123 | 0.488 |
| d 45 | 54.3 | 39.4 | 42.8 | 65.6 | 42.9 | 77.9 | 1.51 | 0.443 | 0.831 | 0.893 | 0.732 |
| IL-6 | | | | | | | | | | | |
| d 10[9] | 255.5 | 98.2 | 244.7 | 177.4 | 94.8 | 313.8 | 1.49 | 0.946 | 0.053 | 0.102 | 0.481 |
| d 45 | 50.2 | 29.7 | 40.8 | 43.4 | 27.8 | 59.5 | 1.49 | 0.698 | 0.369 | 0.551 | 0.823 |
| IL-8 | | | | | | | | | | | |
| d 10 | 767.8 | 430.0 | 867.2 | 564.1 | 797.9 | 698.6 | 1.34 | 0.812 | 0.597 | 0.074 | 0.485 |
| d 45 | 138.5 | 91.5 | 97.4 | 117.2 | 146.8 | 96.9 | 1.34 | 0.869 | 0.287 | 0.683 | 0.854 |
| IL-10 | | | | | | | | | | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| d 10[9] | 267.4 | 98.7 | 259.8 | 182.4 | 95.2 | 303.6 | 1.52 | 0.921 | 0.063 | 0.097 | 0.471 |
| d 45 | 44.5 | 27.1 | 33.7 | 41.0 | 29.3 | 49.5 | 1.52 | 0.655 | 0.686 | 0.711 | 0.864 |
| IL-12 | | | | | | | | | | | |
| d 10 | 777.8 | 801.1 | 838.7 | 673.4 | 836.0 | 878.3 | 1.32 | 0.768 | 0.615 | 0.722 | 0.854 |
| d 45 | 333.4 | 294.0 | 243.1 | 220.2 | 251.0 | 305.2 | 1.32 | 0.500 | 0.581 | 0.674 | 0.258 |
| IL-18 | | | | | | | | | | | |
| d 10[9] | 312.1 | 137.8 | 265.9 | 242.6 | 158.2 | 406.4 | 1.40 | 0.570 | 0.118 | 0.169 | 0.539 |
| d 45 | 75.5 | 48.5 | 49.7 | 66.1 | 52.4 | 74.8 | 1.40 | 0.504 | 0.915 | 0.811 | 0.869 |
| TNFα | | | | | | | | | | | |
| d 10 | 89.7 | 50.9 | 42.0 | 52.5 | 41.9 | 174.9 | 1.32 | 0.001 | 0.025 | 0.523 | 0.175 |
| d 45 | 26.1 | 21.7 | 18.7 | 20.3 | 19.1 | 28.5 | 1.32 | 0.324 | 0.548 | 0.848 | 0.459 |
| TAC, mMol CRE | | | | | | | | | | | |
| d 10 | 585.5 | 594.1 | 618.2 | 625.6 | 605.3 | 611.1 | 32.24 | 0.773 | 0.980 | 0.805 | 0.322 |
| d 45 | 729.6 | 750.0 | 744.1 | 748.9 | 680.4 | 637.0 | 32.24 | 0.008 | 0.456 | 0.695 | 0.836 |
| SOD, U/mL | | | | | | | | | | | |
| d 10[9] | 2.57 | 3.49 | 3.31 | 3.15 | 2.53 | 2.56 | 0.376 | 0.095 | 0.808 | 0.312 | 0.595 |
| d 45 | 3.72 | 4.09 | 3.85 | 3.61 | 3.47 | 3.58 | 0.241 | 0.376 | 0.470 | 0.796 | 0.470 |

[1] A total of 360 pigs (initially 5.4 ± 0.01 kg) were used in a 45-d growth study with 5 pigs per pen and 12 replicates per treatment. Serum cytokine profile was evaluated at Eve Technologies (Calgary, AB, Canada). Data were log10 transformed for statistical analysis and transformed back for the cell mean values reported in this table.

[2] No significant treatment × day interactions ($P > 0.05$).

[3] SOD; superoxide dismutase. TAC; total antioxidant capacity.

[4] Mecadox 2.5 (Phibro; Teaneck, NJ) included at 55 mg/kg of complete feed.

[5] No significant interactive responses ($P > 0.10$) of carbadox and HY were observed.

[6] Increasing HY with no carbadox.

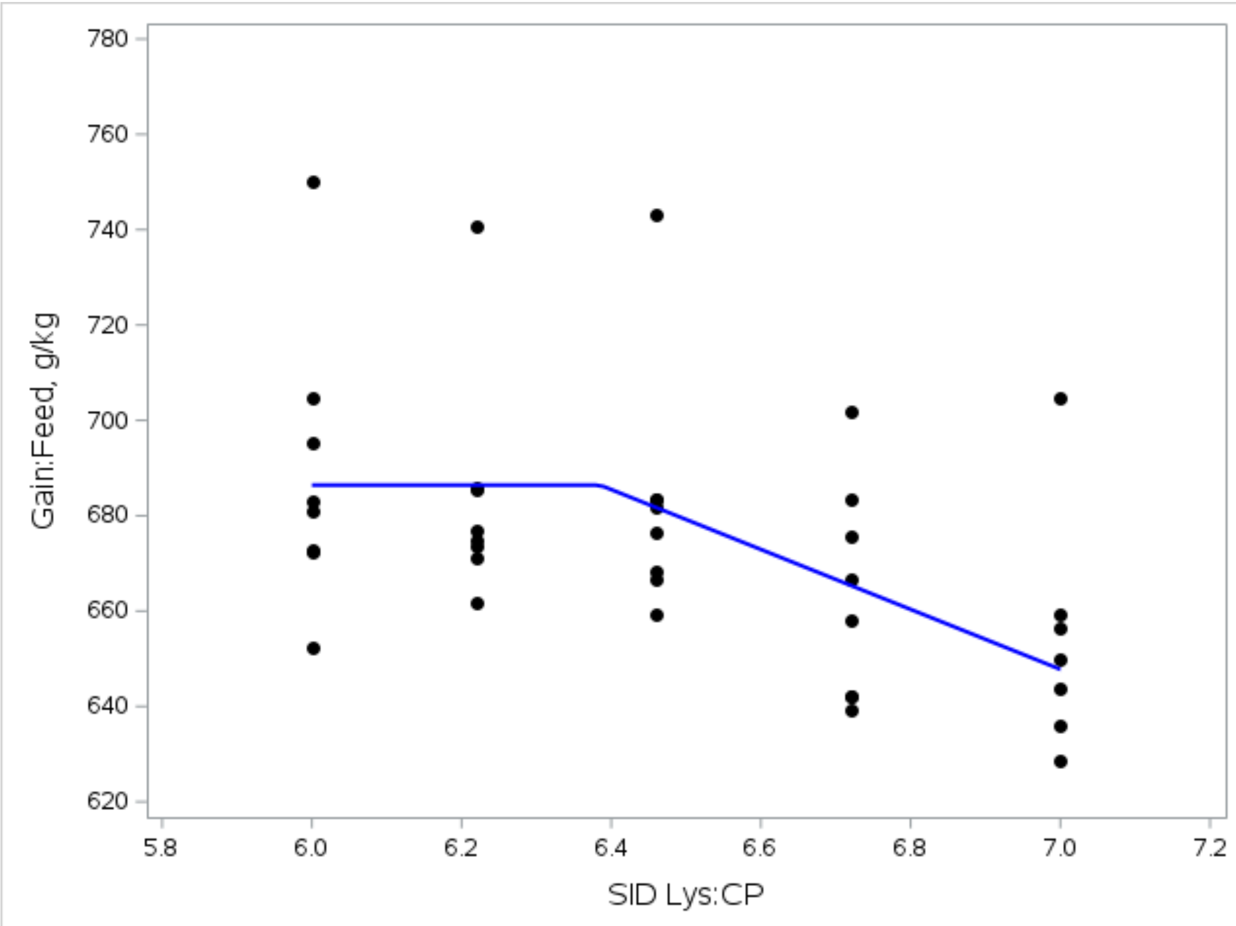
[7] Comparing the mean of 0% HY with and without carbadox vs. the mean of 0.04% HY with and without carbadox.

[8] Comparing the mean of 0% and 0.04% HY with carbadox vs. the mean of 0 and 0.04% HY without carbadox.

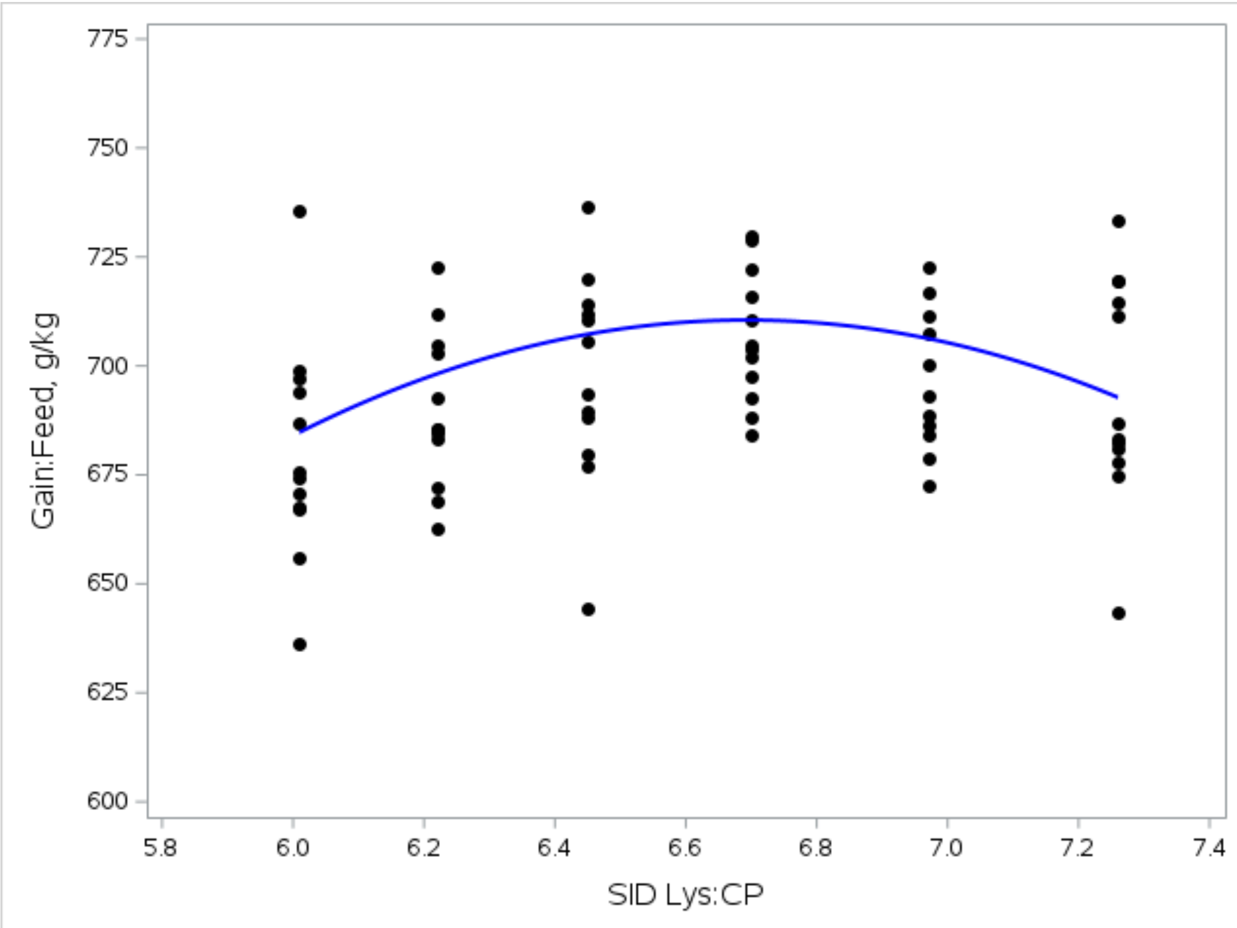
[9] Pairwise comparison of carbadox with 0.04% HY vs. carbadox without HY, $P < 0.10$.

# Appendix A - Effects of SID Lys:CP ratio on feed efficiency

**Appendix Figure A.1.** Influence of SID Lys:CP ratio on feed efficiency in Exp. 2. Breakpoint analysis indicates inflection point was observed at SID Lys:CP of 6.39% in 1.15% SID Lys diets.



**Appendix Figure A.2.** Influence of SID Lys:CP ratio on feed efficiency in diets without DDGS in Exp. 3. Maximum G:F was observed at SID Lys:CP of 6.69%.



**Appendix Figure A.3.** Influence of SID Lys:CP ratio on feed efficiency in diets with 15% DDGS in Exp. 3. Maximum G:F was observed at SID Lys:CP of 6.47%.