Evaluating the effects of specialty protein sources on nursery pig performance and measurement of acid-binding capacity of common nursery pig feed ingredients

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Abstract

The 3 chapters of this thesis involve 1) an evaluation of fermented corn protein and its effects in either high or low branch chain amino acid to leucine ratio diets on nursery pig performance and feed intake preference, 2) an evaluation of the acid-binding capacity of ingredients and complete diets commonly used for weanling pigs, and 3) the influence of anchovy fish meal compared to other protein sources on nursery pig performance. Chapter 1 consisted of 3 experiments which used 880 weaned pigs to evaluate fermented corn protein's effect on nursery pigs. In Exp. 1, fermented corn protein was evaluated as a potential replacement to enzymatically treated soybean meal. In Exp. 2, pigs were fed increasing levels of fermented corn protein with either low or high branch chain amino acid to leucine ratios. In Exp. 3, fermented corn protein and its components were evaluated to measure nursery pig feed intake preference. Results from the studies suggest that fermented corn protein decreases nursery pig performance and increasing branch amino acid to leucine ratio only improves feed efficiency, however, whole stillage solids appear to be the component of fermented corn protein that negatively affect feed intake preference. Chapter 2 measured the acid-binding capacity of common nursery pig feed ingredients and evaluated acid-binding capacity additivity in complete diets. The results of this study suggest a low acid-binding capacity diets can be successfully formulated through careful selection of ingredients. Ingredients, with the exception of calcium carbonate and zinc oxide, appear to be additive in complete diets. Calcium carbonate and zinc oxide's acid-binding capacity contribution in complete diets did not match its value from ingredient analysis. Chapter 3 consisted of two experiments which used 2,502 weaned pigs to determine the influence of anchovy fish meal compared to other protein sources on nursery pig performance. In both

experiments, pigs were fed diets containing one of six vegetable or animal protein sources with Exp. 2 being held in a commercial environment. Protein sources included enzymatically treated soybean meal, spray-dried bovine plasma, fermented soybean meal with or without fish solubles, fish meal with or without fish solubles. In Exp. 1, fish meal with solubles and spray-dried bovine plasma resulted in a 5 to 7% improvement in average daily gain, although results were not significant. However, in Exp. 2, pigs fed fish meal with solubles had decreased growth performance compared to other protein sources.

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Chapter 1 - Evaluation of fermented corn protein and its effects in either high or low branch chain amino acid ration diets on nursery pig performance and feed intake preference

Abstract

Three experiments were conducted to evaluate fermented corn protein (FCP) in nursery pig diets. The removal of non-fermentable components before fermentation of DDGS results in high protein dried distillers grains (HPDDGs). Fermented corn protein is produced when protein and yeast fraction syrup from ethanol production is added back to HPDDGs resulting in a product with up to 50% CP and 2% Lys. In Exp. 1, 350 barrows, initially 6.0 kg, were used to evaluate FCP as a replacement to enzymatically treated soybean meal. Treatments were arranged in a 2 × 2 + 1 factorial with main effects of specialty protein source (FCP or enzymatically treated soybean meal) and level (5 or 10%) or a control diet without any specialty protein source. There were 5 pigs per pen and 14 replications per treatment. From d 0 to 31, pigs fed enzymatically treated soybean meal had improved ($P < 0.05$) ADG and feed efficiency (G:F) compared to pigs fed FCP. In Exp. 2, 350 pigs, initially 12.1 kg, were used to determine the effects of FCP with high or low Ile and Val (Ile+Val):Leu ratio on growth performance. Treatments were arranged in a 2 × 2 + 1 factorial with main effects of FCP level (10 or 20%) and Ile+Val:Leu ratio (low or high) in addition to a corn-soybean meal control diet with 5 pigs per pen and 14 replications per treatment. From d 0 to 21, ADG, ADFI, and G:F worsened (linear, $P < 0.001$) as FCP increased. High Ile+Val:Leu improved ($P < 0.05$) G:F compared to low Ile+Val:Leu. In Exp. 3, 180 pigs, initially 7.7 kg, were used in a feed intake preference trial evaluating various FCP fractions. A total of 6 diet comparisons with 5 pigs per pen and 6 replications per comparison were used. Corn protein sources and fractions used included: FCP, HPDDGs, whole stillage solids (approximately 2/3 of FCP), and thin stillage solids (approximately 1/3 of FCP), and a control diet. Pigs preferred ($P < 0.001$) the control diet by consuming 82.5% of their intake compared with a diet containing FCP. There was no difference ($P > 0.05$) in feed consumption of diets containing whole stillage solids compared to FCP. Pigs preferred ($P = 0.001$) the diet containing thin stillage solids by consuming 75.8% of their intake with this diet compared to the diet containing FCP. In conclusion, feeding FCP decreased growth performance in nursery pigs, but increasing Ile+Val:Leu improved G:F. Diet preference comparisons suggest that whole stillage solids are the component of FCP that leads to reduced feed intake.

Key Words: Branch chain amino acids, enzymatically treated soybean meal, fermented corn protein, growth, nursery pig

Introduction

Young pigs undergo a physiological stress event when they transition from a mostly liquid milk diet to dry cereal-based diet at weaning. Soybean meal in cereal-based diets compound the problem because antigenic proteins in soybean meal, such as glycinin and β-conglycinin, cause a hypersensitive immune response in the gastro-intestinal tract of weaned pigs (Li et al., 1991). This hypersensitivity results in abnormal morphology of the small intestines and reduced absorptive capacity (Li et al., 1991). Therefore, alternative protein sources, such as enzymatically treated soybean meal, are sometimes used to reduce soybean level in the diet of the weaned pig. However, enzymatically treated soybean meal increases diet cost and its impact on growth performance of weanling pigs is inconsistent (Zhou et al., 2011; Ma et al., 2019;

Ruckman et al., 2020). Therefore, alternatives to enzymatically treated soybean meal may be beneficial to swine producers.

Dried distillers grains with solubles (DDGS) is a co-product of the ethanol industry. The removal of non-fermentable components before fermentation of DDGS results in an alternative product often times referred to as high protein dried distillers grains (HPDDGs) which contains approximately 40% crude protein (CP; Sekhon et al., 2015; Cemin et al., 2020). When various protein and yeast fractions of ethanol production are added back to the HPDDGs, it results in a fermented corn protein with up to 50% CP and 2% Lys. Because of its high CP and Lys content, fermented corn protein has the potential to become an excellent replacement for specialty soy protein products such as enzymatically treated soybean meal.

Corn-based ingredients contain high amounts of Leu which could cause potential problems because of an imbalance in branch chain amino acids (BCAA) relative to Lys (Cemin et al., 2019). Excess dietary Leu present in corn-based ingredients can interfere with utilization of the other BCAA, Val and Ile, ultimately increasing their dietary requirement (Harris et al., 2004; Cemin et al., 2019). Increasing the level of other BCAA in diets containing excess Leu has been shown to improve growth performance of finishing pigs (Kerkaert et al., 2021). However, little data is available to determine if a similar strategy of increasing other BCAA will improve performance of nursery pigs fed diets with increased levels of Leu.

Research has shown that feed intake is reduced when pigs are fed diets containing HPDDGs (Yang et al., 2018; Cemin et al., 2021; Rao et al., 2021). Seabolt et al. (2010) reported when pigs are given a choice of HPDDGs or control diets, pigs preferred the control diet even when HPDDGs were included at low levels. However, there is limited research on the effects fermented corn protein and its components have on nursery pig performance and feed intake preference.

The objective of these studies were to evaluate fermented corn protein as a replacement to enzymatically treated soybean meal, the influence of elevated Ile+Val:Leu in fermented corn protein-containing diets on nursery pig growth performance, and to determine the impact of corn protein sources or fractions on nursery pig feed intake preference.

Materials and Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments. Experiment 1 was conducted at the Kansas State University Segregated Early Weaning facility in Manhattan, KS. Experiment 2 and Exp. 3 were conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Experiment 1 was conducted between two identical nursery rooms and a single nursery room was used in Exp. 2 and Exp. 3. All nursery rooms are completely enclosed, environmentally controlled, and mechanically ventilated. For Exp. 1 and 2, each pen contained a 4-hole, dry self-feeder and a nipple waterer to provide *ad libitum* access to feed and water. In Exp. 3, each pen was equipped with two identical 4-hole, dry self-feeders and a single nipple waterer to provide *ad libitum* access to feed and water. For Exp. 1, pens (1.2 × 1.2 m) had tri-bar floors and allowed approximately 0.30 m^2/pig. For Exp. 2 and Exp. 3, pens (1.3 × 1.3 m) had metal tri-bar floors and allowed approximately 0.34 m^2/pig.

Chemical analysis. A sample of fermented corn protein and enzymatically treated soybean meal were submitted to the Agricultural Experiment Station Chemical Laboratory

(University of Missouri-Columbia, Columbia, MO) for total amino acid (AA) analysis (method 982.30; AOAC International, 2006), CP, DM, EE, crude fiber (ANKOM Technology, 2005), ash (method 942.05; AOAC International, 1990), Ca (method 985.01; AOAC International, 1990) and P (method 985.01; AOAC International, 1990) (Table 1). Standardized ileal digestibility coefficients for fermented corn protein were derived from Yang et al. (2020). A sample of fermented corn protein was submitted to the North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND) to determine mycotoxin concentration through extraction in acetonitrile and water followed by chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) detection. Fermented corn protein was found to contain 51 ppb aflatoxin and 16.7 ppm of total fumonisin. All other mycotoxins were below detectable levels.

Experiment 1

Animals and treatment structure. The objective of Exp. 1 was to evaluate fermented corn protein as a replacement to enzymatically treated soybean meal in the diets of newly weaned pigs. A total of 350 barrows (DNA 200 × 400; initially 6.0 ± 0.13 kg BW) were used in a 31-d phase 1 and 2 nursery trial. Pigs were weaned at approximately 21-d of age and placed in pens of 5 pigs based on initial body weight (BW) and pens were randomly allotted to 1 of 5 dietary treatments with 14 replications per treatment.

Five dietary treatments were arranged in a 2 × 2 + 1 factorial with main effects of specialty protein source (fermented corn protein or enzymatically treated soybean meal) and inclusion level (5 or 10%). A standard corn-soybean meal control diet with no fermented corn protein or enzymatically treated soybean meal was also fed. Individual pig weights and feed disappearance were measured on d 10, 17, 24, and 31 to determine average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F).

Pigs were fed phase 1 diets from placement until d 10 and then offered phase 2 diets from d 10 to 31. Diets (Tables 2 and 3) were formulated to contain 1.40% (phase 1) and 1.35% (phase 2) standardized ileal digestible (SID) Lys and met or exceeded nutrient requirement estimates established by the National Research Council (NRC, 2012). The inclusion of L-Lys in the control diet was consistent with the enzymatically treated soybean meal diets, with soybean meal level altered to balance for SID AA. The soybean meal level in the 5 and 10% enzymatically treated soybean meal diets were consistent with the 5 and 10% fermented corn protein diets, respectively. Therefore, to balance for SID AA across all diets, feed-grade AA were increased in the fermented corn protein diets accordingly. Diets were manufactured in pellet form at Provimi North America (Lewisburg, OH).

Experiment 2

Animal and treatment structure. The objective of Exp. 2 was to determine the effects of fermented corn protein inclusion level with high or low Ile and Val (Ile+Val):Leu ratio on nursery pig growth performance. A total of 350 pigs (DNA 600 × 241, initially 12.1 ± 1.72 kg BW) were used in a 21-d nursery trial. Pigs were weaned at approximately 21-d of age and placed in pens of 5 pigs based on initial weight and gender and then fed a common diet. Each pen contained at least 2 barrows and 2 gilts. On d 24 after weaning (d 0 of the trial), pens of pigs were weighed and then allotted to 1 of 5 dietary treatments with 14 replications per treatment. Individual pig weights and feed disappearance were measured on d 10 and 21 to determine ADG, ADFI, and G:F.

Five dietary treatments were arranged in a 2 × 2 + 1 factorial with main effects of fermented corn protein level (0, 10, or 20%) and Ile+Val:Leu ratio (low or high). A standard corn-soybean meal diet acted as the control that also served as 0% inclusion of fermented corn protein. Fermented corn protein was included at the expense of the soybean meal and the high Ile+Val:Leu diets were formed by increasing the inclusion of L-Val and L-Ile. Diets (Table 4) were formulated to contain 1.30% SID Lys and met or exceeded nutrient requirement estimates established by the NRC (2012). The experimental diets were manufactured in meal form at Provimi North America (Lewisburg, OH). The fermented corn protein diets were added at the expense of soybean meal and increased addition of Val and Ile were utilized to increase the high Ile+Val:Leu treatments.

Experiment 3

Animals and treatment structure. The objective of Exp. 3 was to evaluate nursery pig feed intake preference from various corn protein sources or fractions. A total of 180 pigs (DNA 600 × 241), were used in a series of three, 5-d nursery trials with a different set of 60 pigs for each trial. Pigs were weaned at approximately 21-d of age and placed in pens of 5 pigs, each based on initial weight and gender. Each pen contained at least 2 barrows and 2 gilts. The first set of 60 pigs (initially 7.7 ± 0.78 kg BW) were weighed and allotted to 1 of 4 diet comparisons on d 17 after weaning. The second set of 60 pigs (initially 10.3 ± 0.45 kg BW) were weighed and allotted to the same 4 diet comparisons as the first set of pigs (achieving a total of 6 replications/comparison) on d 22 after weaning. The third set of 60 pigs (initially 11.7 ± 0.72 kg BW) were weighed and allotted to 1 of 2 additional diet comparisons (6 replications) on d 27 after weaning.

Diet preparation. To determine feed intake preference, each pen was equipped with two identical feeders containing different diets. Feeders were rotated daily within pen to minimize feeder location bias. The basal diet (Table 5) was manufactured at Hubbard Feeds (Beloit, KS) and divided into 5 batches. Corn protein sources were added and mixed at the Kansas State

University O.H. Kruse Feed and Technology Center (Manhattan, KS) to form 5 experimental diets. The corn protein sources, or fractions utilized in the diets included: fermented corn protein, high protein dried distillers grains (HPDDGs), whole stillage solids (approximately 2/3 content of fermented corn protein), and thin stillage solids (approximately 1/3 content of fermented corn protein). Fermented corn protein and HPDDGs were included in the diet at 15% as a replacement to corn. Whole stillage solids and thin stillage solids were included in the diet at 10% and 5%, respectively, as a replacement to corn to match its contribution in the fermented corn protein diet. A standard corn-soybean meal diet with 15% added corn to match the contribution of corn protein sources used in other diets was used as the control. A total of 6 diet comparisons were evaluated by utilizing all 5 experimental diets. The diet comparisons for the first and second groups of pigs included: 1) Control vs. fermented corn protein; 2) Whole stillage solids vs. fermented corn protein; 3) Thin stillage solids vs. fermented corn protein; and 4) HPDDGs vs. fermented corn protein. The diet comparisons utilized for the third set of pigs included: 5) Control vs. whole stillage solids; and 6) Control vs. thin stillage solids. Feed disappearance was measured at the end of each 5-d trial to determine feed disappearance from each diet per pen.

Statistical analysis

In Exp. 1 and 2, data were analyzed as a randomized complete block design with body weight serving as a blocking factor and pen as the experimental unit. Block was included in the model as a random effect. In Exp. 1, linear and quadratic polynomial contrasts of increasing levels of fermented corn protein or enzymatically treated soybean meal were tested as well as any interactions considering the control diet as the 0% inclusion level. In Exp. 2, linear and quadratic polynomial contrasts of increasing levels of fermented corn protein were tested considering the control diet as the 0% inclusion level. High and low Ile+Val:Leu ratios were also tested as well as any interactions with increasing levels of fermented corn protein.

In Exp. 3, data were analyzed as a completely randomized design with feeder within pen as the experimental unit. Pen and group were included in the model as a random effect for the first four diet comparisons. Pen was included in the model as a random effect for comparison 5 and 6. Data are reported as the mean difference in average daily feed disappearance between the two diets within each comparison as a percentage of the total feed consumed for each diet within each comparison. All data were analyzed using the lmer package using the RStudio environment [Version 4.0.2 (2020-06-22), R Core Team, R Foundation for Statistical Computing, Vienna, Austria]. Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results

Chemical analysis. Fermented corn protein, as expected, contained approximately 50% CP and 2% Lys. As anticipated, enzymatically treated soybean meal was higher in CP and Lys compared to fermented corn protein containing 55% and 3%, respectively. Fermented corn protein was 6 percentage units higher in EE than enzymatically treated soybean meal. Fermented corn protein was also higher in the BCAA Leu compared to enzymatically treated soybean meal as expected.

Experiment 1

There were no interactions observed ($P > 0.10$) between protein source and inclusion level for any growth performance criteria (Table 6). In phase 1 (d 0 to 10), there were no protein source or inclusion level main effects ($P > 0.10$) for BW, ADG, ADFI, or G:F.

In phase 2 (d 10 to 31), pigs fed fermented corn protein had decreased ($P = 0.045$) ADG compared to pigs fed enzymatically treated soybean meal. Pigs that were fed increasing levels of fermented corn protein had decreased (linear, $P \leq 0.011$) ADG and ADFI with no differences in G:F. Pigs fed increasing levels of enzymatically treated soybean meal tended to have decreased (linear, $P = 0.068$) ADFI.

Overall (d 0 to 31), pigs fed enzymatically treated soybean meal had improved ($P \leq 0.034$) BW, ADG, and G:F compared to pigs fed fermented corn protein with no differences in ADFI. Pigs fed increasing levels of fermented corn protein had decreased (linear, $P \leq 0.026$) BW, ADG, and ADFI with no differences in G:F. Increasing the level of enzymatically treated soybean meal had no impact on performance relative to the control diet.

Experiment 2

There were no BCAA × fermented corn protein interactions ($P > 0.05$) throughout the duration of the experiment (Table 7). From (d 0 to 10) and overall (d 0 to 21), pigs fed fermented corn protein diets with high Ile+Val:Leu had improved ($P \leq 0.019$) G:F compared to pigs fed fermented corn protein diets with low Ile+Val:Leu. From d 0 to 10, d 10 to 21, and overall (d 0 to 21), pigs fed diets containing increasing levels of fermented corn protein had worsened (linear, $P \leq 0.020$) BW, ADG, ADFI, and G:F.

Experiment 3

For comparison 1, pigs preferred ($P < 0.001$) the control diet over the diet containing fermented corn protein with 82.5% of the intake coming from the control diet (Table 8). For comparison 2, there was no difference ($P > 0.05$) in feed consumption of diets containing whole stillage solids or fermented corn protein. For comparison 3, pigs preferred ($P < 0.001$) the diet containing thin stillage solids by consuming 75.8% of the total pen intake with this diet compared to the diet containing fermented corn protein. For comparison 4, pigs tended (P = 0.067) to prefer the diet containing HPDDGs (59.7% of their intake) compared to the fermented corn protein diet. For comparison 5, pigs preferred (P < 0.001) the control diet by consuming 86.7% of their total pen intake with this diet compared with the diet containing whole stillage solids. For comparison 6, pigs preferred (P = 0.028) the control diet compared to the diet containing thin stillage solids with 56.9% of total pen intake

Discussion

Soybean meal is usually the main protein source in pig diets. However, newly weaned pigs can experience transient hypersensitivity to soybean meal proteins that cause damage to the microvilli in the small intestine and reduced absorptive capacity (Friesen et al., 1993). This allergic reaction has led to the use of alternative protein sources, such as alternative soy or corn protein, in the diets of nursery pigs to replace soybean meal. To improve production efficiency, ethanol plants are removing the fibrous components of corn before fermentation to produce HPDDGs (Yang et al., 2019). Adding components from the fermentation process back to the HPDDGs produces a fermented corn protein product that is high in CP and Lys. Fermented corn protein is 10 percentage units greater in CP than HPDDGs and therefore could be a potential alternative to soy protein products such as enzymatically treated soybean meal.

In Exp. 1, fermented corn protein was evaluated as a potential replacement to enzymatically treated soybean meal in early nursery diets. Enzymatically treated soybean meal is produced by treating conventional soybean meal with enzymes such as proteases and carbohydrases (Goebel and Stein, 2011). The resulting product is considered a high-quality protein source for weaned pigs because of its reduced antinutritional factors compared to conventional soybean meal (Li et al., 1991; Yang et al., 2007). Previous studies have reported increasing levels from 7 to 21% of enzymatically treated soybean meal in the diet decreased overall body weight, daily gain, and feed intake in nursery pigs (Jones et al., 2017; Ruckman et al., 2020). In contrast, Zhou et al. (2011) observed an improvement in growth performance from feeding increasing enzymatically treated soybean meal from 5 to 15% of the diet. The results from the current study indicate enzymatically treated soybean meal does not influence growth performance compared to a standard corn soybean meal-based diet.

To our knowledge there is limited research on the effects of fermented corn protein on nursery pig performance. In the current study, fermented corn protein worsened ADG and G:F compared to enzymatically treated soybean meal without influencing ADFI. This suggests that fermented corn protein decreased efficiency of nutrient utilization compared to enzymatically treated soybean meal. These results indicate fermented corn protein cannot act as a complete replacement for enzymatically treated soybean meal in nursery pig diets. Increasing the level of fermented corn protein included in nursery diets also decreased ADG and ADFI of nursery pigs. It has been documented antagonistic effects of excess Leu can result in decreased Val and Ile utilization negatively affecting the growth performance (Yang et al., 2019). However, diets in the current study were formulated to contain adequate or above adequate levels of Ile and Val across all diets to ameliorate any negative effects of excess Leu.

Corn co-products have disproportionally greater content of the BCAA Leu, compared to the other BCAA, Ile and Val (NRC, 2012). An excess of one BCAA will lead to increased catabolism of all BCAA (Harper et al., 1984). Therefore, inclusion of corn co-products in nursery diets may cause an imbalance in BCAA resulting in decreased growth performance. Kwon et al. (2022) reported diets with increasing SID Leu:Lys ratio decreased daily gain and feed intake in growing pigs. Cemin et al. (2019) suggested increasing levels of Ile and Val can prevent decreased growth performance in pigs fed diets containing excess Leu. Kerkaert et al. (2021) was able to improve growth performance of finishing pigs fed excess Leu diets by supplementing the other BCAA at levels greater than typically utilized to meet the pig's requirement. In contrast, Kwon et al. (2021) reported the addition of Ile and Val alone or in combination did not affect growth performance in diets containing excess Leu in finishing pigs. However, the effects of increased Ile and Val in diets containing excess Leu on nursery pig performance is still relatively unclear (Kerr et al., 2004; Gloaguen et al., 2011; Millet et al., 2015). In the current study, increasing the Ile+Val:Leu ratio only improved feed efficiency in nursery pigs. This agrees with Kwon et al. (2021) where they observed no difference in daily gain or intake from feeding high Ile and Val in growing pigs. However, these results are in contrast with the results of Kerkaert et al. (2021), where the authors observed an improvement in daily gain and intake with no effect on feed efficiency from feeding high Ile or Val in growing-finishing pigs. Ultimately, from Exp. 2, the decrease in daily gain with addition of fermented corn protein was driven by a combination of decreased feed intake and utilization. Feeding higher amounts of Ile and Val with a diet containing excess Leu was able to recover some feed utilization but did not improve feed intake.

Feed intake can be influenced by many factors such as feed composition, temperature, disease, sex, genetics, and palatability of feed ingredients (Ellis and Augspurger, 2001; Clouard and Val-Laillet, 2014). Seabolt et al. (2010) reported that diets with increasing HPDDGs decreased feed preference in pigs, suggesting its palatability may reduce feed intake and growth performance. However, fermented corn protein differs from HPDDGs because of the addition of protein and yeast fractions from ethanol production. Whole stillage solids represent the HPDDGs component of fermented corn protein, whereas thin stillage solids are the protein and yeast components. It is unknown which fraction of fermented corn protein resulted in the reduced feed intake in nursery pigs based on the results from Exp. 1 and 2. Thus, Exp. 3 compared feed intake preference from fermented corn protein, its components, a HPDDGs source, as well as a control diet with no added corn protein source.

Pigs preferred the control diet compared to the fermented corn protein diet which agrees with the results from Exp. 1 and 2. Similarly, pigs also preferred the control diet to the diet containing whole stillage solids. However, when pigs were offered either fermented corn protein or whole stillage solids diets, there was no preference observed. In contrast, pigs preferred the thin stillage solids diet compared to the diets containing fermented corn protein. These results suggest the whole stillage solids are the component of fermented corn protein that negatively affect feed intake and subsequently daily gain. Even though pigs did prefer the control diet compared to the diet containing thin stillage solids, the difference in daily feed disappearance was much less than when comparing the control diet to the diet with whole stillage solids. Therefore, the HPDDGs component of fermented corn protein likely caused the decreased feed intake, similar to the results of Seabolt et al. (2010). Further research is warranted to determine the effects thin stillage solids (protein and yeast fractions) have on nursery pig growth performance.

Fermented corn protein was found to have 51 ppb of aflatoxin and 16.7 ppm of total fumonisin. This would provide 10.2 ppb of aflatoxin in diets containing 20% fermented corn protein used in Exp. 2 which is below the maximum tolerance level of 300 ppb (United States Food and Drug Administration, 1994). This would also provide 3.3 ppm of total fumonisin in the diet which is below the guidance level of 20 ppm for swine diets (U.S. Food & Drug

Administration, 2001). Therefore, mycotoxin levels present in fermented corn protein would not be expected to diminish performance in all experiments.

In summary, the results of Exp. 1 indicate that utilizing fermented corn protein as a replacement to enzymatically treated soybean meal decreased early nursery pig growth performance. The results of Exp. 2 indicate that increasing concentrations of fermented corn protein decreased growth performance and increasing Ile+Val:Lys did not eliminate the reduction in feed intake, but feed efficiency was improved when Ile+Val:Leu increased in the diet. Results from Exp. 3 indicate the whole stillage solids fraction is likely the component of fermented corn protein that negatively affects pig feed intake and growth performance.

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Table 1.1 Chemical analysis of fermented corn protein and enzymatically treated soybean meal Exp. 1, 2 and 3 (as-fed basis)

Item, %	Fermented corn protein	Enzymatically treated soybean meal
DM	92.22	93.69
CP	50.28	55.10
EE	7.05	1.04
Crude fiber	7.10	5.55
Ash	2.75	6.73
Ca	0.137	3.30
P	0.552	0.691
Amino acids		
Ala	3.67	2.40
Arg	2.21	3.93
Asp	3.25	6.14
Cys	1.02	0.82
Glu	8.59	9.91
Gly	1.86	2.36
His	1.40	1.44
Ile	2.09	2.71
Leu	6.30	4.31
Lys	1.77	3.18
Met	1.19	0.76
Phe	2.66	2.84
Pro	4.09	2.80
Ser	2.14	2.46
Thr	1.85	2.18
Trp	0.33	0.71
Tyr	2.00	1.92
Val	2.68	2.81

[1] A sample of fermented corn protein and enzymatically treated soybean meal was collected, homogenized, subsampled, and submitted to the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO) for proximate analysis and amino acid profile.

Table 1.2 Phase 1 diet composition, Exp. 1 (as-fed basis)[1]

		Fermented corn protein, %		Enzymatically treated soybean meal, %	
Item	Control	5	10	5	10
Ingredients, %					
Corn	36.60	37.15	37.75	37.40	38.20
Soybean meal (46.5% CP)	31.40	25.60	19.80	25.60	19.75
Whey powder	25.00	25.00	25.00	25.00	25.00
Fermented corn protein	---	5.00	10.00	---	---
Enzymatically treated soybean meal	---	---	---	5.00	10.00
Choice white grease	2.50	2.50	2.50	2.50	2.50
Limestone	0.95	1.00	1.05	0.98	0.98
Monocalcium phosphate (21% P)	1.70	1.68	1.65	1.70	1.70
Salt	0.50	0.50	0.50	0.50	0.50
L-Lys HCl	0.35	0.46	0.58	0.35	0.35
DL-Met	0.20	0.19	0.18	0.20	0.20
L-Thr	0.16	0.18	0.20	0.16	0.16
L-Trp	0.02	0.04	0.05	0.02	0.02
L-Val	0.09	0.11	0.12	0.08	0.07
L-Ile	---	0.04	0.08	---	---
Zinc oxide	0.39	0.39	0.39	0.39	0.39
Vitamin-trace mineral premix	0.15	0.15	0.15	0.15	0.15
Total	100	100	100	100	100

Table 1.2 (cont.)

Item	Control	Fermented corn protein, % 5	Fermented corn protein, % 10	Enzymatically treated soybean meal, % 5	Enzymatically treated soybean meal, % 10
Calculated analysis					
SID amino acids, %					
Lys	1.40	1.40	1.40	1.40	1.40
Ile:Lys	62	62	62	63	63
Leu:Lys	110	116	121	111	113
Met:Lys	34	34	34	34	35
Met and Cys:Lys	56	56	56	56	56
Thr:Lys	63	63	63	63	63
Trp:Lys	19.0	19.0	19.0	19.0	19.0
Val:Lys	70	70	70	70	70
His:Lys	33	34	35	33	33
Total Lys, %	1.54	1.56	1.58	1.53	1.53
NE, kcal/kg	2,806	2,795	2,787	2,776	2,743
SID Lys:NE, g/Mcal	4.99	5.01	5.03	5.05	5.11
CP, %	21.5	21.6	21.6	21.6	21.7
Ca, %	0.89	0.89	0.89	0.89	0.89
STTD P, %	0.63	0.63	0.63	0.63	0.63

[1] Diets were fed from approximately 6.0 to 7.0 kg.

Table 1.3 Phase 2 diet composition, Exp. 1 (as-fed basis)[1]

		Fermented corn protein, %		Enzymatically treated soybean meal, %	
Item	Control	5	10	5	10
Ingredients, %					
Corn	51.85	52.45	53.00	53.60	55.00
Soybean meal (46.5% CP)	31.00	25.20	19.40	25.20	19.35
Whey powder	10.00	10.00	10.00	10.00	10.00
Fermented corn protein	---	5.00	10.00	---	---
Enzymatically treated soybean meal	---	---	---	5.00	10.00
Choice white grease	2.50	2.50	2.50	2.50	2.50
Limestone	1.00	1.05	1.10	1.03	1.03
Monocalcium phosphate (21% P)	1.80	1.78	1.75	1.83	1.83
Salt	0.50	0.50	0.50	0.50	0.50
L-Lys HCl	0.42	0.54	0.65	0.42	0.42
DL-Met	0.19	0.19	0.18	0.20	0.20
L-Thr	0.20	0.22	0.24	0.20	0.20
L-Trp	0.04	0.05	0.06	0.04	0.04
L-Val	0.10	0.12	0.14	0.10	0.09
L-Ile	---	0.06	0.10	0.02	0.02
Zinc oxide	0.25	0.25	0.25	0.25	0.25
Vitamin-trace mineral premix	0.15	0.15	0.15	0.15	0.15
Total	100	100	100	100	100

Table 1.3 (cont.)

Item	Control	Fermented corn protein, %		Enzymatically treated soybean meal, %	
		5	10	5	10
Calculated analysis					
SID amino acids, %					
Lys	1.35	1.35	1.35	1.35	1.35
Ile:Lys	61	61	61	59	59
Leu:Lys	111	116	121	109	109
Met:Lys	35	35	35	35	35
Met and Cys:Lys	56	56	56	56	55
Thr:Lys	63	63	63	63	63
Trp:Lys	19.0	19.0	19.0	19.0	19.0
Val:Lys	70	70	70	70	70
His:Lys	34	35	36	33	33
Total Lys, %	1.49	1.51	1.53	1.49	1.48
NE, kcal/kg	2,818	2,806	2,795	2,782	2,751
SID Lys:NE, g/Mcal	4.79	4.81	4.83	4.85	4.91
CP, %	21.2	21.1	21.1	20.6	20.6
Ca, %	0.83	0.83	0.83	0.83	0.83
STTD P, %	0.56	0.56	0.56	0.56	0.56

[1] Diets were fed from approximately 7.0 to 15.9 kg.

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

Item	Fermented corn protein[2]:		Low Ile+Val:Leu		High Ile+Val:Leu	
		0%	10%	20%	10%	20%
Ingredients, %						
Corn		63.85	63.80	63.80	63.65	63.50
Soybean meal (46.5% CP)		32.60	22.40	12.20	22.40	12.25
Fermented corn protein		---	10.00	20.00	10.00	20.00
Limestone		0.88	0.95	1.03	0.95	1.03
Monocalcium phosphate (21% P)		0.90	0.85	0.80	0.84	0.78
Salt		0.50	0.50	0.50	0.50	0.50
L-Lys HCl		0.38	0.57	0.75	0.57	0.75
DL-Met		0.16	0.13	0.09	0.13	0.09
L-Thr		0.21	0.22	0.24	0.22	0.24
L-Trp		0.05	0.07	0.09	0.07	0.09
L-Val		0.10	0.11	0.12	0.19	0.28
L-Ile		---	---	---	0.08	0.15
Vitamin premix with phytase[2]		0.25	0.25	0.25	0.25	0.25
Trace mineral premix		0.15	0.15	0.15	0.15	0.15
Total		100	100	100	100	100

Table 1.4 (cont.)

			Low Ile+Val:Leu		High Ile+Val:Leu	
Item	Fermented corn protein:	0%	10%	20%	10%	20%
Calculated analysis						
SID amino acids, %						
Lys		1.30	1.30	1.30	1.30	1.30
Ile:Lys		62	58	54	64	65
Leu:Lys		117	131	145	131	145
Met:Lys		34	34	33	34	33
Met and Cys:Lys		57	57	57	57	57
Thr:Lys		65	65	65	65	65
Trp:Lys		20.5	20.5	20.6	20.5	20.6
Val:Lys		74	74	74	80	86
His:Lys		36	40	43	40	43
Ile+Val:Leu		116	100	88	109	104
Total Lys, %		1.45	1.50	1.54	1.50	1.54
NE, kcal/kg		2,740	2,718	2,696	2,723	2,703
SID Lys:NE, g/Mcal		4.74	4.78	4.82	4.78	4.81
CP, %		21.5	22.0	22.6	22.1	22.8
Ca, %		0.68	0.68	0.68	0.68	0.68
STTD P, %		0.46	0.46	0.46	0.46	0.46

[1] Diets were fed from approximately 12.1 to 24.7 kg.

[2] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.13% STTD P with 1,250 FTU/kg.

Table 1.5 Basal diet composition Exp. 3 (as-fed basis)[1]

Item	Basal diet[2]
Ingredients, %	
Corn	56.95
Soybean meal (46.5% CP)	25.95
Whey powder	10.00
Fish meal	2.50
Choice white grease	1.00
Limestone	0.75
Monocalcium phosphate (21% P)	0.60
Salt	0.50
L-Lys HCl	0.48
DL-Met	0.20
L-Thr	0.21
L-Trp	0.04
L-Val	0.15
Zinc oxide	0.25
Vitamin premix with phytase[3]	0.25
Trace mineral premix	0.15
Total	100

Table 1.5 (cont.)

Item	Basal diet[2]
Calculated analysis	
SID amino acids, %	
Lys	1.35
Ile:Lys	55
Leu:Lys	111
Met:Lys	36
Met and Cys:Lys	57
Thr:Lys	63
Trp:Lys	18.8
Val:Lys	70
His:Lys	34
Total Lys, %	1.49
NE, kcal/kg	2,504
SID Lys:NE, g/Mcal	5.40
CP, %	20.7
Ca, %	0.74
STTD P, %	0.49

[1] Diets were fed from approximately 7.7 to 13.7 kg.

[2] The basal diet was approximately 85% of the experimental diets. Corn protein sources were included at 15% of the experimental diets. Corn protein source inclusion of experimental diets included: Control (15% corn), fermented corn protein (15% fermented corn protein), whole stillage solids (10% whole stillage solids; 5% corn), thin stillage solids (5% thin stillage solids; 10% corn), and HPDDGs (15% HPDDGs).

[3] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.13% STTD P with 2,060 FTU/kg.

Table 1.6 Interactive effects of protein source and inclusion level on nursery pig performance, Exp. 1[1]

		Fermented corn protein		Enzymatically treated soybean meal			P^2 =				
								Fermented corn protein		Enzymatically treated soybean meal	
Item	Control	5%	10%	5%	10%	SEM	Protein source[3]	Linear	Quad	Linear	Quad
BW, kg											
d 0	6.0	6.0	6.0	6.0	6.0	0.04	0.998	0.968	0.834	0.946	0.979
d 10	7.1	7.0	6.9	7.0	7.2	0.13	0.233	0.230	0.855	0.730	0.386
d 31	16.5	15.4	15.4	16.0	16.0	0.28	0.029	0.004	0.079	0.166	0.417
Phase 1 (d 0 to 10)											
ADG, g	114	99	91	100	119	11.7	0.176	0.116	0.823	0.752	0.218
ADFI, g	142	128	123	124	139	8.8	0.500	0.104	0.689	0.773	0.116
G:F	0.79	0.77	0.70	0.80	0.83	0.055	0.103	0.201	0.746	0.556	0.862
Phase 2 (d 10 to 31)											
ADG, g	439	400	401	426	417	10.6	0.045	0.011	0.103	0.129	0.886
ADFI, g	558	508	514	532	527	12.0	0.119	0.010	0.051	0.068	0.464
G:F	0.79	0.79	0.78	0.80	0.79	0.009	0.153	0.572	0.912	0.810	0.291
Overall (d 0 to 31)											
ADG, g	331	300	299	319	318	9.3	0.034	0.013	0.150	0.298	0.635
ADFI, g	419	381	386	398	398	10.6	0.161	0.026	0.090	0.153	0.412

G:F	0.79	0.79	0.78	0.80	0.80	0.008	0.010	0.154	0.774	0.403	0.476

[1] A total of 350 pigs (initial BW of 6.0 ± 0.04 kg) were used with 5 pigs per pen and 14 pens per treatment.

[2] There were no significant protein source × inclusion level interactions observed ($P > 0.05$). Linear and quadratic analysis within protein source considered the control diet representing an inclusion level of 0%.

[3] Comparison of fermented corn protein and enzymatically treated soybean meal excluding the control.

Table 1.7 Interactive effect of fermented corn protein and Ile+Val:Leu ratio on nursery pig performance, Exp. 2[1]

Ile+Val:Leu		Low		High			P^2 =			
Fermented corn protein:	0%	10%	20%	10%	20%	SEM	BCAA	10 vs. 20%	Linear	Quad
BW, kg										
d 0	12.1	12.0	12.0	12.1	12.1	1.55	0.691	0.912	0.810	0.967
d 10	17.9	17.2	16.4	17.7	16.3	2.11	0.607	0.007	0.001	0.438
d 21	26.7	25.1	23.1	25.6	23.2	2.47	0.554	< 0.001	< 0.001	0.303
d 0 to 10										
ADG, g	577	498	414	552	421	53.9	0.071	< 0.001	< 0.001	0.075
ADFI, g	794	779	734	789	717	74.4	0.873	0.014	0.020	0.266
G:F	0.73	0.64	0.57	0.70	0.59	0.019	0.019	< 0.001	< 0.001	0.374
d 10 to 21										
ADG, g	799	723	635	719	621	42.0	0.621	< 0.001	< 0.001	0.667
ADFI, g	1,184	1,101	1,054	1,100	995	81.3	0.242	0.004	< 0.001	0.877
G:F	0.68	0.66	0.61	0.65	0.63	0.014	0.362	< 0.001	< 0.001	0.316
Overall (d 0 to 21)										
ADG, g	693	615	531	640	526	47.0	0.449	< 0.001	< 0.001	0.197
ADFI, g	998	946	903	952	863	77.8	0.410	0.002	< 0.001	0.670
G:F	0.70	0.65	0.59	0.67	0.61	0.009	0.017	< 0.001	< 0.001	0.134

[1]A total of 324 pigs (initial BW of 12.1 ± 1.55 kg) were weaned at approximately 21-d of age and placed in pens of 5 pigs based on initial weight and gender and fed a common diet. On d 24 after weaning (d 0 of the trial), pens of pigs were weighed and then allotted to 1 of 5 dietary treatments with 14 replications per treatment.

[2] There were no significant BCAA × fermented corn protein interactions or interactive tendencies observed ($P > 0.05$). The BCAA and fermented corn protein columns compare the low and high Ile+Val:Leu formulation and the 10/20% inclusion of fermented corn protein and does not include the control treatment. The Linear and Quad columns include the control (0%), 10, and 20% fermented corn protein averaged across low/high Ile+Val:Leu.

Table 1.8 Effect of corn protein source on feed intake preference in nursery pigs[1]

Item	Control	Fermented corn protein	Whole stillage solids	Thin stillage solids	HPDDGs	SEM	*P* =
Comparison 1[2]	82.5	17.5	---	---	---	3.24	< 0.001
Comparison 2[2]	---	48.0	52.0	---	---	2.99	0.377
Comparison 3[2]	---	24.2	---	75.8	---	2.45	< 0.001
Comparison 4[2]	---	40.3	---	---	59.7	6.66	0.067
Comparison 5[3]	86.7	---	13.3	---	---	4.07	< 0.001
Comparison 6[3]	56.9	---	---	43.1	---	3.81	0.028

[1] A total of 180 pigs were used in a 15-d preference trial with 5 pigs per pen and 6 replications per comparison. Three 5-d preference trials were evaluated with a different set of 12 pens and 60 pigs per trial. Feeders were rotated once daily within each pen to eliminate any feeder location bias. Data reported as feed disappearance, % is the percentage of total feed intake for each treatment within a comparison.

[2] Comparison 1 to 4 were utilized in the first (d 17 to 22 after weaning) and second (d 22 to 27 after weaning) set of 60 pigs. Comparisons were randomly assigned to 12 pens within a trial for a total of 6 replications per comparison.

[3] Comparisons 5 and 6 were utilized in the third (d 27 to 32 after weaning) set of 60 pigs. Comparisons were randomly assigned to 12 pens for a total of 6 replications per comparison.

Chapter 2 - Evaluation of the acid binding capacity of ingredients and complete diets commonly used for weanling pigs

Abstract

Some ingredients bind more acid in the stomach than others which can increase gastric pH in weaned pigs causing decreased protein digestion and allow pathogenic micro-organisms to proliferate. The objective of this experiment was to measure acid-binding capacity at a pH of 4 (ABC-4) of common nursery ingredients and determine additivity in diets. Ingredient categories included: cereal grains, vegetable proteins, animal proteins and milk, vitamin premixes and minerals, amino acids, and fiber sources. A 0.5 g sample of each ingredient was suspended in 50 mL of distilled deionized water and titrated with 0.1 N hydrochloric acid. Sample ABC-4 was calculated as the amount of acid in milliequivalents (meq) required to lower 1 kg to a pH of 4. Cereal grains were found to have lower ABC-4 compared to other ingredients. Vegetable proteins had higher ABC-4 with more variation than cereal grains. Soybean meal (SBM) had an ABC-4 of 602 ± 28.2 meq. Soy protein concentrate and enzymatically treated soybean meal (ESBM) had higher ABC-4 compared to SBM while fermented soybean meal (FSBM) was lower. Zinc oxide (ZnO) and calcium carbonate (CaCO3) had the highest ABC-4 among all ingredients with values of 21,863 ± 598.7 and 18,384 ± 769.7 meq, respectively. Following ingredient analysis, a series of diets were analyzed to determine additivity by comparing the differences between calculated and analyzed ABC-4. All diets analyzed had lower ABC-4 than calculated values; however, analyzed ABC-4 increased along with calculated values across diets. The first series of diets were arranged in a 2 × 5 factorial consisting of increasing CaCO3 with or without ZnO. There was a ZnO × CaCO3 interaction (P = 0.020) for difference between calculated and analyzed ABC-4. Within the interaction, differences between calculated and analyzed ABC-4 increased (linear, $P < 0.001$) as CaCO3 increased in diets without ZnO, but not in diets with ZnO. The second series of diets analyzed consisted of different levels of SBM with either FSBM or ESBM included at 5% of the diet. Differences between calculated and analyzed values were not different between treatments ($P = 0.640$). In conclusion, perfect ABC-4 additivity in diets was not found due to lower analyzed than calculated values; however, analyzed ABC-4 still increased as calculated values increased. This data suggests diet ABC-4 can be adjusted through selection of ingredients but feeding trials are needed to determine its impact on pig performance.

Key Words: Acid-binding capacity, ingredients, diets, nursery pig

Introduction

The postweaning period is a stressful and critical moment of the young pig's life and much interest has been directed toward positively influencing the gastrointestinal tract (GIT) to maximize lifetime production and health status (Pluske, 2016). Newly weaned pigs undergo a dramatic change in diet composition from a highly palatable and digestible milk diet to a dry cereal-based diet. Stomach acid secretion is low in the suckling pigs and the main source of acidity comes from bacterial fermentation of lactose to lactic acid (Cranwell et al., 1968). However, at weaning the amount of lactic acid in the stomach is reduced compared to a suckling pig (Yen, 2001). In North America, it is common for pigs to be weaned between 17 and 21 days with a relatively underdeveloped GIT (McGlone and Johnson, 2003). Pigs' hydrochloric acid production is limited until the pig reaches 7 to 8 weeks of age (Pluske, 2016; Pealin et al., 2020). Switching pigs to a solid diet at weaning may lead to an increase in gastric pH as high as 5.0 for several days post-weaning (Kidder and Manners, 1978). Low stomach pH is important for protein digestion and when stomach pH increases above 3.5, pepsin activity rapidly declines (Longland 1991; Zijlstra et al., 1996; Yen, 2001; Pluske, 2003). Increased gastric pH allows opportunistic pathogens to survive and compromise the digestive tract leading to clinical infection, disease, and possible death (Bolduan et al., 1988).

Poor acidification of the stomach can be attributed to the high acid-binding capacity (ABC) of feed ingredients (Batonon-Alavo et al., 2016). The concept of ABC involves manipulating the stomach's acidity by incorporating low acid-binding ingredients (Lawlor et al., 2005). Therefore, the use of low acid binding ingredients (avoidance of high acid-binding ingredients) in the diets of newly weaned pigs could be utilized to maintain an acidic environment in the stomach and improve feed utilization. Lawlor et al. (2005) evaluated ingredients common in early nursery swine diets and reported minerals had the highest ABC values, specifically calcium carbonate and zinc oxide (ZnO). A study by Warner et al. (2022) reported an improvement in daily weight gain and feed efficiency with no effect on feed intake as calcium carbonate percentage in the diet decreased post-weaning. These data are an indication of potentially better feed utilization in diets with lower ABC; however, additional research is warranted to understand if the response was due to low ABC, changing calcium carbonate levels, or other factors. Lawlor et al. (2005) reported vegetable proteins, such as soybean-meal, were found to have a relatively high ABC when compared to cereal grains and make up a large percentage of early nursery pig diets, thus heavily influencing the ABC. Therefore, partially replacing soybean meal in the diets of nursery pigs with an ingredient low in ABC can not only reduce the instance of transient hypersensitivity but could also allow more efficient nutrient utilization from reduced ABC. Ingredients evaluated by Lawlor et al. (2005) are European sourced and ABC may not be fully representative of ingredients used in North America.

Furthermore, little research has been conducted on additivity of ABC ingredient values in complete diets which is important for ABC diet formulation.

The ABC of complete diets can be predicted if each ingredient value in the diet is known. Batonon-Alvao et al. (2016) reported additivity of ABC from mixing wheat, soybean meal, and dicalcium phosphate and found calculated and analyzed values to be similar. However, it is common for early nursery diets to contain a variety of specialty ingredients, such as animal proteins and dairy products, to improve palatability and digestibility. Further investigation is warranted to determine additivity of ABC in complex early nursery diets and allow successful manipulation of ABC in complete diets to potentially improve utilization of nutrients and subsequent performance and GIT health of nursery pigs.

Therefore, the objective of this experiment was to evaluate the ABC-4 of common North American feed ingredients used in diets of nursery pigs and determine if ABC-4 values are additive when mixed together to form complete diets.

Materials and Methods

General

The experiment was conducted at the Kansas State University Swine Nutrition Laboratory in Manhattan, KS. Ingredients commonly used in early nursery diets were obtained from commercial sources.

Protocol

All ingredient samples and diets were ground to achieve a consistent particle size of approximately 400 µm using a Rancilio Rocky Doserless Grinder (Rancilio Group; Villastanza, Italy). Approximately 100 g of each sample was then placed into a sample bag and stored at -

20°C until ABC-4 was determined using a modified procedure established by Lawlor et al. (2005). Modifications include beaker size, pH meter, and ending equilibrium criteria. Half a gram sample of each ingredient and diet was weighed on a weigh boat then suspended in 50 mL of distilled de-ionized water. All samples, apart from minerals, were placed in a 100 mL beaker with a magnetic stir bar. Minerals were placed in a 250 mL beaker with a magnetic stir bar because of a large amount of acid required to lower pH. The beaker was placed on a stir plate and samples were stirred until suspended in solution. Initial pH of each sample was recorded using a Mettler Toledo SevenCompact pH/Ion S220 meter. Each day of analysis, the pH meter was calibrated using a Fisher calibrated buffer solution. Titrations were performed by the addition of 0.1 N hydrochloric acid (HCl) in increments of 0.1 to 5.0 mL depending on ingredient and stage of titration. Once a stable pH of 4.00 ± 0.04 was reached, ABC-4 was calculated as the amount of acid in milliequivalents (meq) required to achieve pH of 4 for 1 kg of sample. Samples with an initial pH less than 4 were titrated using sodium hydroxide (NaOH) to raise the pH. Sample pH equilibrium was determined was once the meter indicated stabilization.

Ingredient analysis

Ingredients were obtained from various commercial feed mills in the United States or ingredient suppliers (Table 1). Ingredients were grouped into categories: cereal grains, vegetable proteins, animal proteins and milk, vitamin premixes and minerals, amino acids (AA), and fiber sources. Each sample was analyzed three separate times to observe any variation within source and ABC-4 was calculated as the average of the triplicate analysis. This value was then used in statistical analysis. Sources of ingredients listed are an indication of manufacturing location and may not be representative of collection location.

Cereal grains included: corn (IA, KS, MI, MN, PA), soft red winter wheat (IN and MI), white wheat (MI), sorghum (KS), barley (OH, PA), whole oats (KS, MI), oat groats (KS), dried distillers grains with solubles (IA, KS, MN), and cereal blend (Quincy Farm Products, Quincy, IL). Vegetable proteins included: soybean meal (IA, KS, MI, MN, PA), expelled soybean meal (OH), soy protein concentrate (Xsoy, CJ Bio, Seoul, South Korea; SoyTide, CJ Bio, Seoul, South Korea), fermented soybean meal (MEpro, Prairie AquaTech, Brookings, SD; Fermex, Purina Animal Nutrition, Shoreview, MN; Proplex T, ADM Animal Nutrition, Quincy, IL), fermented soy isolate (AX3, TripleA, Hornsyld, Denmark), enzymatically treated soybean meal (HP 300, Hamlet Protein, Findlay, OH), and high protein dried distillers grains with solubles (NexPro, Poet, Sioux Falls, SD; Protomax, ICM, Colwich, KS). Animal proteins and milk included: fish meal (TASA, Lima, Peru; Omega Protein, Houston, TX), spray-dried bovine plasma (APC, Ankeny, IA), poultry meal (AV-E Digest, XFE Products, Des Moines, IA) spray-dried whey powder (KS), whey permeate (KS), and crystalline lactose (MN). Amino acids included: L-Lys (Ajinomoto, Eddyville, IA), DL-Met (Adisseo, Alpharetta, GA), L-Thr (Ajinomoto, Eddyville, IA), L-Trp (Ajinomoto, Eddyville, IA), L-Val (Ajinomoto, Eddyville, IA), L-Ile (Ajinomoto, Eddyville, IA). Vitamin premixes and minerals included: calcium carbonate (IA, KS, MI), ZnO (Zinc Nacional, Monterrey, Mexico), dicalcium phosphate (PCS, Northbrook, IL; MN), monocalcium phosphate (PCS, Northbrook, IL), calcium propionate (NutraBlend, Neosho, MO), vitamin premix (Swine Nutrition Guide, Kansas State University, Manhattan, KS; manufacturer: DSM, Exton, PA), trace mineral premix (Swine Nutrition Guide, Kansas State University, Manhattan, KS; manufacturer: Nutra Blend, Neosho, MO), vitamin trace mineral premix (MN), manganese (MN), sodium chloride (KS), sodium metabisulfite (MI), and choline chloride (KS). Fiber source included: beet pulp shreds (KS, MI).

Early nursery diets

Two different early nursery diets were formulated to determine if ABC-4 values for ingredients are additive by comparing calculated ABC-4 with analyzed values. All diets were mixed in the Kansas State Applied Swine Nutrition Laboratory (Manhattan, KS). Ingredients used to formulate diets were from the same samples used in the ingredient analysis to reduce any variation in ABC from ingredient source. The same procedures as individual ingredients were used to determine analyzed ABC-4 of complete feeds.

The first series of diets analyzed were phase 1 nursery diets intended for pigs weighing 5 to 7 kg. A total of 10 diets were arranged in a 2 × 5 factorial with increasing levels of calcium carbonate with or without the inclusion of 3,000 mg/kg of Zn from ZnO (Table 2). Calcium carbonate and ZnO diets were selected because of their high ABC-4. Diets were formulated to contain 0, 0.45, 0.90, 1.35, and 1.80% calcium carbonate at the expense of corn. Diets without ZnO contained 110 mg/kg of Zn from the trace-mineral premix.

The second series of diets analyzed were phase 2 nursery diets intended for pigs weighing 7 to 12 kg. A total of 3 diets were formed with different levels of soybean meal, feed-grade AA, and specialty soy proteins products (Table 3). The two soy products included fermented soybean meal (MEpro, Prairie AquaTech, Brookings, SD) and enzymatically treated soybean meal (HP 300, Hamlet Protein, Findlay, OH) because of their low and high ABC-4, respectively. The first diet consisted of low soybean meal and high feed-grade AA with 5% fermented soybean meal. The second diet consisted of low soybean meal and high feed-grade AA with 5% enzymatically treated soybean meal. The third diet consisted of high soybean meal and low feed-grade AA with 5% enzymatically treated soybean meal. These diets were selected because of the large difference in ABC-4 for different protein sources.

Statistical analysis

All data analysis was performed using the RStudio environment (Version 1.3.1093, RStudio, Inc., Boston, MA) using R programming language [Version 4.0.2 (2020-06-22), R Core Team, R Foundation for Statistical Computing, Vienna, Austria]. For ingredient analysis, descriptive statistics were generated using the summary function with value reported as average of sample means ± standard deviation of sample means. For calcium carbonate and ZnO diet analysis, data were analyzed as a completely randomized design using the lm function with difference between calculated and analyzed ABC-4 as the experimental unit. The main effects of ZnO and calcium carbonate, as well as their interactions, were tested. For soybean meal and specialty protein analysis, data were analyzed as a completely randomized design using the lm function with difference between calculated and analyzed ABC-4 as the experimental unit. The main effects of diet were tested. For testing of all hypotheses, differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results

Ingredient analysis

The mean ABC-4 values of each ingredient are reported as the average of sample means ± standard deviation of sample means (Table 1). Cereal grains with respect to other ingredients were found to have a low ABC-4. Corn was found to have an ABC-4 of 84 ± 18.6 meq, with barley having a similar value to corn. Dried distillers grains with solubles (DDGS), cereal blend, oat products, and sorghum were among the highest of ABC-4 within cereal grains and wheat products were found to have the lowest values.

Vegetable proteins were among two ingredient categories with the most variation between ingredients and sources. Soybean meal was found to have an ABC-4 of 602 ± 28.2 meq. Other soy products had a wide range of ABC-4 values. Enzymatically treated soybean meal and soy protein concentrate had higher ABC-4 than soybean meal, whereas fermented soybean meal had a lower value. However, there was great variation between soy protein concentrate and fermented soybean meal with standard deviations of 165.0 and 100.0 meq, respectively. Fermented soy isolate had an ABC-4 of -13 meq that had to be titrated with NaOH. Finally, high protein dried distillers grains (HPDDGs) had a low ABC-4 relative to other vegetable proteins.

Animal proteins and milk collectively, with the exception of crystalline lactose, were higher in ABC-4 than vegetable proteins. Fish meal had the highest ABC-4 of 1,380 ± 150.9 meq most likely due to its high Ca content. Spray-dried bovine plasma, poultry meal, spray-dried whey powder, and whey permeate had lower values than fish meal, but still relatively high values compared to other ingredients tested. Crystalline lactose had the lowest ABC-4 of all animal proteins and milk with a value of 53 meq.

Vitamin premixes and minerals had the highest ABC-4 between categories and had the most variation, as expressed by the standard deviations. Calcium carbonate and ZnO had the highest ABC-4 of 18,384 ± 769.7 and 21,863 ± 598.7 meq, respectively. These were also the two ingredients with the highest ABC-4 overall. The vitamin premix, trace-mineral premix, vitamin trace mineral premix, manganese, calcium propionate, and dicalcium phosphate also had high ABC-4, but did not reach the level of calcium carbonate and ZnO. Monocalcium phosphate, sodium chloride, choline chloride, and sodium metabisulfite had low values among vitamin premixes and minerals.

Amino acids were more consistent compared to other ingredient categories. All feed-grade AA analyzed had an ABC-4 between 83 and 200 meq, with L-Lys and L-Ile having the lowest and highest value, respectively.

The fiber source tested was another ingredient low in ABC-4 compared to the other categories. Beet pulp shreds were found to have an ABC-4 of 151 ± 25.3 meq.

Complete diets

Calcium carbonate and ZnO. Calculated and analyzed ABC-4 increased with the amount of calcium carbonate and ZnO added in the diet. The difference between calculated and analyzed ABC-4 was used to test ZnO and limestone's additivity when mixed with other ingredients to form complete diets.

There was a ZnO × calcium carbonate interaction observed ($P = 0.015$) for difference between calculated and analyzed ABC-4 (Table 4; Figure 1). Within the interaction, the difference between calculated and analyzed ABC-4 increased (linear, $P < 0.001$) as calcium carbonate levels increased in diets without ZnO. The difference between analyzed and calculated ABC-4 at 0% calcium carbonate was 29 meq without ZnO. This difference increased along with the addition of calcium carbonate with the largest difference of 185 meq observed at 1.80% calcium carbonate. However, the difference between calculated and analyzed ABC-4 did not significantly change ($P > 0.05$) as calcium carbonate increased in the diet when ZnO was present. The lowest and highest differences were observed at 0 and 1.35% calcium carbonate with the difference between analyzed and calculated ABC-4 being 116 and 157 meq, respectively.

Soybean meal and specialty protein. The inclusion of enzymatically treated soybean meal and high levels of soybean meal increased the calculated and analyzed ABC-4 of complete diets compared to a diet with fermented soybean meal and low levels of soybean meal (Table 5). Similar to the first series of diets analyzed, all diets had lower analyzed values than calculated values. All three diets had similar differences between calculated and analyzed ABC-4 values. As a result, the differences between calculated and analyzed ABC-4 of all three diets was not significant (P = 0.640). The average difference between calculated and analyzed ABC-4 was 107 meq.

Discussion

Early nursery diets typically contain a variety of ingredients to improve palatability and digestibility. Ingredients that have a high ABC-4 will bind more acid in the stomach of the newly weaned pig (Lawlor et al., 2005). The binding of acid in the stomach of newly weaned pigs can raise the stomach pH which is detrimental to the overall performance and health (Bouldan et al., 1988; Yen, 2001). A high gastric pH inhibits protein digestion and is associated with proliferation of harmful micro-organisms that can result in severe cases of post-weaning diarrhea leading to diminished performance or death (Kidder and Manners, 1978; Longland, 1991; Partanen and Mroz 1999).

The present study aimed to determine the ABC-4 of common early nursery ingredients utilized in North America. Buffering capacity is also a way to measure an ingredient's ability resist a change in pH. Buffering capacity is calculated by diving the ABC-4 by the total change in pH, therefore buffering capacity can also be calculated from the values reported in Table 1. This study also aimed to determine additivity of ingredient values for successful diet formulation regarding ABC-4. Overall, analyzed ingredient ABC-4 were generally similar to values reported by Lawlor et al. (2005) with the differences present most likely due to processing procedures and geographical location. Specifically, plant-based ingredient's ABC-4 can be influenced by season, soil fertility, rate of fertilization, and stage of maturity which affects their ion concentration (Jasaitis et al., 1987).

Calcium carbonate and ZnO had the highest ABC-4 of all ingredients analyzed. Lawlor et al. (2005) also reported high ABC-4 for calcium carbonate and ZnO, but the authors values were approximately 5,452 and 5,542 meq lower than the current study, respectively. Zinc oxide's high ABC-4 can raise a complete diet's value even though it is included at low percentages. Although a high ABC-4 is thought to be detrimental to the newly weaned pigs, it is likely the beneficial antimicrobial properties of ZnO outweigh the potential negative effects of increased ABC-4 of the diet. Zinc ions prevent attachment and translocation of pathogenic bacteria, but there are growing concerns of its use at pharmacological levels (Huang et al., 1999; Starke et al., 2014). Low ABC-4 diets may improve gut health of pigs to provide some of the benefit of Zn at pharmacological levels. Calcium carbonate had the second highest ABC-4 of all ingredients analyzed. Bouldan et al. (1988) suggested limiting the calcium (Ca) content in the diets of early nursery pigs to improve health but was unsure of the growth response to the limited mineral content. However, Warner et al. (2022) reported low calcium carbonate levels in the diets of early nursery pigs improved gain with no effect on intake indicating better feed utilization. Additional research needs to be done to determine if low ABC-4 diets are beneficial to early nursery pig's health and subsequent performance.

Soybean meal is generally used as the main protein source in diets for pigs in North America. Similar to the current study, Lawlor et al. (2005) reported soybean meal to have an ABC-4 of 642 ± 51.1 meq and Batonon-Alavo et al. (2016) reported a value of 610 ± 6.8 meq. Soybean meal was found to have a high ABC-4 relative to corn and these are the two main ingredients in many swine diets in the US. Therefore, the diets containing higher soybean meal levels will have higher ABC-4 values. Soybean meal can also vary in concentrations of Ca depending on the source (Huang et al., 2017). The ABC-4 of the diet may be affected more so if the soybean meal source is high in Ca. The inclusion of feed-grade amino acids can lower the soybean meal inclusion and thus decrease ABC-4 of the diet. Amino acid values for the current study were all less than the reported values from Lawlor et al. (2005), but only by an average of 53 meq for Lys, Met, Thr, and Trp. Specialty protein sources can also be utilized to partially replace soybean meal and decrease ABC-4. However, specialty protein products have varying ABC-4 values. Soy protein concentrate and enzymatically treated soybean meal had higher ABC-4 than conventional soybean meal, whereas fermented soybean meal had lower values. Based on vegetable protein analysis, it appears fermentation of soybean meal reduces ABC-4. The sample of fermented soy isolate was found to have a negative ABC-4. This product not only has a low ABC-4 from the fermentation process but is also acid washed further reducing its value. Therefore, a specialty protein source that has a low ABC-4, such as fermented soybean meal or fermented soy isolate, could be utilized to decrease the ABC-4 of the diet.

All cereal grains in the current study had ABC-4 values similar to the values reported by Lawlor et al. (2005), although the mean ABC-4 with the exception of oat products were slightly lower in the current study. Cereal grains, such as corn, were lower in ABC-4 compared to other ingredients analyzed. Wheat was found to have the lowest ABC-4 of all cereal grains. Wheat-based diets can lower ABC-4 from 40 to 150 meq depending on the inclusion percentage compared to corn-based diets. Therefore, wheat-based diets would be the most useful cereal grain to minimize the ABC-4 of complete diets.

Animal proteins and milk had higher ABC-4 compared to other ingredients with the exception of crystalline lactose. The high ABC-4 is most likely related to the Ca and protein contents in these ingredients (Jasaitis et al., 1987; Bouldan et al., 1988; Lawlor et al., 2005). Even though these ingredients would appear to raise ABC-4, their effects on the diet ABC-4 are not as great because of the ingredients they often replace. Inclusion of fish meal in the diet reduces the need for calcium carbonate and soybean meal which also contribute to ABC-4 (Rojas and Stein, 2012). However, fish meal was an ingredient with a high standard deviation for ABC-4. Fish meal from the current study had a much higher ABC-4 than the value reported by Lawlor et al. (2005) of 738 ± 219.3 meq. As a result, the source and composition of fish meal should be considered when evaluating ABC-4 of a diet. Spray-dried bovine plasma, whey powder, and whey permeate are highly digestible protein sources for young pigs and their ABC-4 are similar to soybean meal (Kim et al., 2012; Rojas and Stein, 2012). Therefore, the ingredients will not raise, or lower ABC-4 drastically compared to using soybean meal. However, using crystalline lactose as opposed to whey powder or whey permeate as the lactose source could lower the overall diet ABC-4.

Phosphorus is important for energy metabolism, nucleic acid synthesis, bone formation and mineralization of early nursery pigs (Berndt and Kumar, 2009). Dicalcium phosphate contains one anion per molecule whereas monocalcium phosphate contains two (Lima et al., 1991). As a result, monocalcium phosphate contains more negative charges that lower its ABC-4 relative to dicalcium phosphate. Therefore, depending on the P source used, ABC-4 can change. Dicalcium phosphate was found to have a much higher ABC-4 than monocalcium phosphate as expected. Utilizing monocalcium phosphate as the P source can reduce the ABC-4 in the diet.

Premixes are added to swine diets to ensure the pig is receiving adequate vitamin and mineral levels. Trace mineral premixes had a higher ABC-4 relative to other ingredients because of the cations that are associated with the minerals present. Vitamin premixes analyzed had an

ABC-4 even greater than the trace mineral premixes. The vitamins present in a vitamin premix do not significantly contribute to its ABC-4, compared with the carrier, namely calcium carbonate. Therefore, ABC-4 of vitamin premixes can change depending on the source and level of carrier used.

Feed additives often represent a small percentage of the total diet; however, their composition is important to understand how it will affect the ABC-4 of the diet. Feed additives that contain high amounts of minerals, and more specifically Ca, can increase the diet ABC-4 even at small inclusions. Therefore, feed additives should not be overlooked when formulating a diet to a low ABC-4 to potentially improve weaned pig performance.

In both diet sets analyzed, the analyzed ABC-4 was less than the calculated values using the ingredient analysis. Therefore, it cannot be concluded that ingredients are perfectly additive in complete diets. It is unclear why analyzed values do not match calculated values, but the inclusion of calcium carbonate and presence of ZnO appear to contribute to this discrepancy. Calcium carbonate's ABC-4 contribution in complete diets may be overestimated when using individual ingredient values. Further investigation revealed once calcium carbonate is less than 2% of a diet its ABC-4 contribution is decreased. The results indicate an ABC-4 of about 8,000 meq would appear to be more appropriate for calcium carbonate when ZnO is not present in the diets to have calculated values align more closely with analyzed values. However, this assumes that all other ingredient ABC-4 values in the diet are perfectly additive. The variation of ABC-4 in ingredients and additivity of other ingredients used should be considered as well. Complete diet analysis also indicates ZnO is not directly additive in complete diets and its contribution to ABC-4 appears to depend on the calcium carbonate level. However, to our knowledge there is no mechanism between ZnO and calcium carbonate that would drive this interaction. It is also possible these ingredients are interacting with an additional ingredient or ingredients within the diet resulting in the discrepancy. Therefore, further investigation is warranted to understand additivity of calcium carbonate and ZnO and any potential interactions that may be occurring to influence ABC-4.

Acid-binding capacity-4 in complete diets were able to be successfully manipulated by including different levels of soybean meal, feed-grade AA, and specialty protein sources. When soybean meal was high in the diet, the ABC-4 increased. The inclusion of enzymatically treated soybean meal also increased ABC-4 compared to diets containing fermented soybean meal. However, analyzed ABC-4 were all lower than calculated values, similar to the results of the calcium carbonate and ZnO diets. As previously stated, the reason for the discrepancy between calculated and analyzed ABC-4 requires further investigation.

In conclusion, complete additivity of ingredients can’t be concluded and warrants further investigation. Reduced ABC-4 of the diet can still be successfully manipulated through careful selection of ingredients. Feeding strategies such as low calcium carbonate levels, low soybean meal inclusion (high feed-grade AA), and use of low ABC-4 ingredients can be used to target a low ABC-4 in complete diets. Targeting a low ABC-4 in the diets of nursery pigs immediately post-weaning may alleviate problems in the early nursery stage such as poor growth, impaired GIT health, and mortality. However, further investigation is warranted to determine the optimal ABC-4 of early nursery pigs of a specific weight range to optimize performance.

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Table 2.1 Acid-binding capacity at pH 4 (ABC-4) of ingredients common in early nursery diets[1]

Item:	N[2]	Average initial pH	Average ABC-4 (meq)
Cereal grains			
Corn	5	6.50	84 ± 18.6
Sorghum	2	7.04	110 ± 14.1
Red winter wheat	2	6.70	43 ± 4.7
White wheat	2	6.67	60 ± 0.0
Barley	2	5.49	77 ± 4.7
Whole oats	3	6.33	122 ± 23.4
Oat groats	2	6.44	107 ± 37.7
Dried distillers grains with solubles	3	4.85	147 ± 43.7
Cereal Blend	1	6.08	107
Vegetable protein			
Soybean-meal	6	6.98	602 ± 28.2
Expelled SBM	1	7.13	567
Soy protein concentrate[4]	2	7.36	737 ± 165.0
Fermented SBM[5]	3	4.69	207 ± 100
Fermented soy isolate[6]	1	3.95	-13
Enzymatically treated SBM[7]	1	6.29	753
HPDDGs[8]	2	4.87	100 ± 84.9
Animal proteins and milk			
Fish meal[9]	2	6.32	1,380 ± 150.9
Spray-dried bovine plasma[10]	1	6.99	713
Poultry meal[11]	1	6.74	1,007
Spray-dried whey powder	1	6.29	440
Whey permeate	1	6.16	520
Crystalline Lactose	1	7.09	53
Vitamins and minerals			
Calcium carbonate	3	9.62	18,384 ± 769.7
Zinc oxide	3	9.55	21,863 ± 598.7
Monocalcium phosphate	1	4.21	73

Dicalcium phosphate	3	5.54	2,693 ± 848.8
Calcium propionate	1	9.11	9,240
Sodium chloride	2	6.00	15 ± 5.0
Vitamin premix	2	6.99	10,767 ± 613.8
Trace mineral premix	2	5.37	7,867 ± 264.0
Vitamin trace mineral premix	1	4.67	1,727
Manganese	1	8.22	2,347
Sodium metabisulfite	1	4.00	0
Choline chloride	1	5.21	40
Amino acids			
L-Lys	2	5.76	83 ± 4.7
DL-Met	2	5.54	137 ± 4.7
L-Thr	2	5.43	160 ± 0.0
L-Trp	1	5.11	120
L-Val	1	5.53	193
L-Ile	1	5.76	200
Fiber source			
Beet pulp	3	5.57	151 ± 25.3

[1] Acid-binding capacity-4 was determined by the amount of 0.1 N HCl required to lower the initial pH of a sample to a stable pH of 4.00 ± 0.04. Each sample was analyzed three times and value reported is average of sample means ± standard deviation of sample means.

[2] Number of samples analyzed, with each sample analyzed in triplicate.

[3] Buffering capacity is calculated as ABC-4 divided by the total change in pH.

[4] Xsoy, CJ Bio, Seoul, South Korea; SoyTide, CJ Bio, Seoul, South Korea.

[5] MEpro, Prairie AquaTech, Brookings, SD; Fermex, Purina Animal Nutrition, Shoreview, MN; Proplex T, ADM Animal Nutrition, Quincy, IL.

[6] AX3, TripleA, Hornsyld, Denmark

[7] HP 300, Hamlet Protein, Findlay, OH.

[8] NexPro, Poet, Sioux Falls, SD; Protomax, ICM, Colwich, KS.

[9] TASA, Lima, Peru; Omega Protein, Houston TX.

[10] APC, Ankeny, IA.

[11] AV-E Digest, XFE Products, Des Moines, IA.

Table 2.2 Calcium carbonate titration with or without ZnO diet composition[1]

		No ZnO					Added ZnO				
Item	CaCO3:	0%	0.45%	0.90%	1.35%	1.80%	0%	0.45%	0.90%	1.35%	1.80%
Corn		52.50	52.05	51.60	51.15	50.70	52.10	51.65	51.20	50.75	50.30
Soybean meal		15.60	15.60	15.60	15.60	15.60	15.60	15.60	15.60	15.60	15.60
Whey powder		20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Enzymatically treated SBM		5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Fish meal		2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Bovine plasma		2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Calcium carbonate		---	0.45	0.90	1.35	1.80	---	0.45	0.90	1.35	1.80
Monocalcium phosphate		0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Salt		0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-Lys-HCl		0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
DL-Met		0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
L-Thr		0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
L-Trp		0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
L-Val		0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Vitamin premix with phytase[2]		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
Zinc oxide		---	---	---	---	---	0.40	0.40	0.40	0.40	0.40
Total		100	100	100	100	100	100	100	100	100	100

Calculated ABC-4, meq[3]	316	398	481	563	645	403	486	568	650	733

[1] A total of 10 diets were formulated to contain increasing levels of calcium carbonate with or without 3,000 ppm of Zn from ZnO. Diets without ZnO contained 110 ppm of Zn from the trace mineral premix. Calcium carbonate was added to the diet at the expense of corn. Diets were formulated for pigs weighing 5.0 to 7.0 kg of BW.

[2] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.13% STTD P with 750 FTU/kg.

[3] Individual sample ABC-4 values were used for calculations and not ingredient mean values.

Table 2.3 Soybean meal and specialty protein diets[1]

Item Diet:	Low SBM, Fermented SBM	Low SBM, Enzymatically treated SBM	High SBM, Enzymatically treated SBM
Corn	60.69	58.48	52.43
Soybean meal	20.42	22.78	29.49
Whey powder	10.00	10.00	10.00
Fermented SBM[2]	5.00	---	---
Enzymatically treated SBM[3]	---	5.00	5.00
Calcium carbonate	0.75	0.80	0.78
Monocalcium phosphate	1.05	0.90	0.80
Salt	0.58	0.55	0.55
L-Lys-HCl	0.51	0.51	0.30
DL-Met	0.21	0.20	0.15
L-Thr	0.20	0.20	0.10
L-Trp	0.04	0.03	---
L-Val	0.15	0.15	---
Vitamin premix with phytase[4]	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15
Total	100	100	100
Calculated ABC-4, meq[5]	352	408	443

[1] A total of three diets were formulated to contain different levels of soybean meal and synthetic amino acids. Two different soy products were included in the diet based on their analyzed ABC-4 values. The diets were formulated for pigs weighing 7.0 to 12.0 kg of BW

[2] MEpro, Prairie AquaTech, Brookings, SD.

[3] HP 300, Hamlet Protein, Findlay, OH.

[4] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.13% STTD P with 1,250 FTU/kg.

[5] Individual sample ABC-4 values were used for calculations and not ingredient mean values.

0

Table 2.4 Evaluation of calculated vs. analyzed ABC-4 on increasing calcium carbonate with or without the presence of ZnO[1]

	No ZnO					Added ZnO				
Calcium carbonate:	0%	0.45%	0.90%	1.35%	1.80%	0%	0.45%	0.90%	1.35%	1.80%
Calculated ABC-4 (meq)	316	398	481	563	645	403	486	568	650	733
Analyzed ABC-4 (meq)[2]	287±11.5	333±23.1	353±23.1	393±11.5	460±20.0	287±11.5	340±20.0	433±11.5	493±11.5	600±20.0
Difference[3]	29[c]	65[c]	128[b]	170[a]	185[a]	116[b]	146[ab]	135[b]	157[ab]	133[b]

[1] A total of 10 diets were analyzed in a 2 × 5 factorial design with increasing levels of calcium carbonate at the expense of corn with or without 3,000 ppm of Zn from ZnO. Each diet was sampled and analyzed three times.

[2] Analyzed ABC-4 values is reported as the diet average from 3 samples ± standard deviation within diet.

[3] ZnO × calcium carbonate interaction (P = 0.015). Dose effect of calcium carbonate when ZnO not included at pharmacological levels, linear P < 0.001. Dose effect of calcium carbonate when ZnO included at pharmacological levels, linear P = 0.175. Main effect of calcium carbonate (linear, P < 0.001). Main effect of ZnO (P = 0.009). SEM = 9.9

1

2

Table 2.5 Evaluation of calculated vs. analyzed ABC-4 of diets formulated with different protein sources and levels of soybean meal[1]

Item	Low SBM, fermented SBM[2]	Low SBM, enzymatically treated SBM[3]	High SBM, enzymatically treated SBM[3]	SEM	*P* =
Calculated ABC-4 (meq)	403	457	487	---	---
Analyzed ABC-4 (meq)[4]	300 ± 20.0	353 ± 11.5	373 ± 11.5	---	---
Difference	103	104	114	8.6	0.637

[1] A total of three diets were analyzed three times with different levels of soybean meal and synthetic amino acids. Two different soy products were used based on their analyzed ABC-4. Each diet was sampled and analyzed three times

[2] MEpro; Prairie Aquatech; Brookings, SD.

[3] HP 300; Hamlet Protein; Findlay, OH.

[4] Analyzed ABC-4 values are reported as the diet average from 3 titration ± standard deviation within diet.

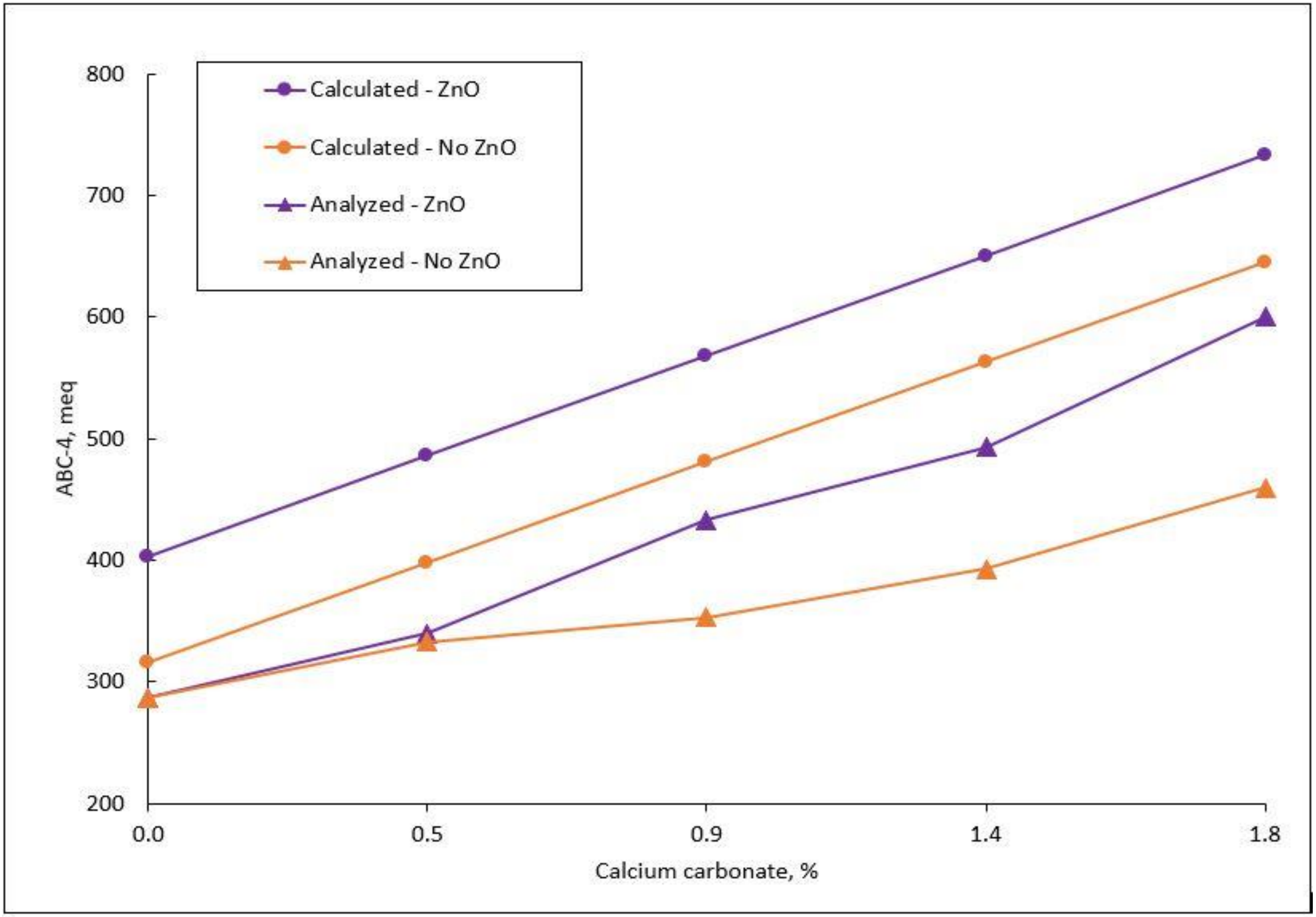

Figure 2.1. Line graph of calculated vs. analyzed ABC-4 on increasing calcium carbonate with or without the presence of ZnO

Chapter 3 - Evaluation of anchovy fish meal and fish solubles compared to other specialty protein sources on nursery pig performance

Abstract

Soybean meal is the predominant protein source used in nursery pig diets; however, it contains antinutritional factors that limit its inclusion rate. Therefore, highly palatable, specialty protein sources such as fish meal or fish derived products can be added to nursery diets to encourage feed intake and promote growth. Two experiments were conducted to evaluate anchovy fish meal and fish solubles compared to other specialty protein sources in nursery pig diets. In Exp. 1, 330 pigs, initially 4.9 kg, were utilized in a university research environment to determine nursery pig growth performance and fecal consistency. At weaning, pigs were randomly allotted to 1 of 6 dietary treatments with 4 or 5 pigs per pen and 12 replications per treatment. Dietary treatments consisting of different protein sources added based on relative SID Lys content included enzymatically treated soybean meal (ESBM), spray-dried bovine plasma (SDBP), fermented soybean meal, fermented soybean meal enriched with 6.5% fish solubles, fish meal, and fish meal enriched with 6.5% fish solubles. At weaning, pigs were fed phase 1 diets for 12 d followed by phase 2 diets from d 12 to 25. Following phase 2, all pigs were fed a common diet for an additional 15 d. In all weigh periods and overall, there were no growth performance differences between treatments. There was a treatment × day interaction (P = 0.003) observed for fecal dry matter (DM) driven by the improvement of pigs fed SDBP from d 12 to 25. Pigs fed SDBP had the lowest fecal DM percentage on d 12 and highest on d 25. In Exp. 2, 2,172 pigs, initially 5.2 kg, were used in a commercial environment to evaluate nursery pig growth performance, morbidity, and mortality. At weaning, pens of pigs were allotted to 1 of 6 dietary treatments based on initial weight and weaning date with 25 or 27 pigs per pen and 14 replications per treatments across two rooms. Dietary treatments were the same as in Exp. 1. Pigs were fed phase 1 diets for 7 d followed by phase 2 from d 7 to 21. Following phase 2, pigs were fed a common diet for an additional 21 d. In the experimental period (d 0 to 21), pigs fed fish meal and SDBP had increased (P = 0.004) ADG compared to pigs fed fish meal with solubles. In summary, both experiments indicate anchovy fish meal can be utilized in nursery pig diets without decreasing growth performance; however, the discrepancy between experiments when fish meal is enriched with fish solubles requires further investigation.

Key Words: Fish meal, fish solubles, protein source, soybean meal, nursery pig

Introduction

Low feed intake is a common issue for newly weaned pigs that can lead to weight loss, morbidity, and mortality. Soybean meal is the predominate protein source used in nursery pig diets but contains antinutritional factors that limit its inclusion rate in diets immediately after weaning. These antinutritional factors cause a hypersensitive immune response in the gastro-intestinal tract of weaned pigs causing diarrhea and decreased performance (Li et al., 1991; Zhang et al., 2003). Therefore, highly palatable, and nutrient dense vegetable or animal proteins are added to nursery diets to meet the pig's amino acid (AA) requirement, encourage feed intake, and promote growth (Jones et al., 2018). Fish meal has balanced AA, vitamins and minerals, and omega 3 fatty acids that provide immunological benefits making it a high-quality protein source (Church and Kellems, 1998; Li et al., 2014).

The main species utilized in the fish meal and fish oil industry include mackerels, capelins, blue whitings, anchovies, menhaden, herring, sardine, and sandeels (Péron et al., 2010). These species of fish are small, bony, and oily fish that are typically not suitable for human consumption. On the other hand, fish meal can be manufactured by utilizing fish by-products associated with processing of various edible human fishery products or produced from fish specifically harvested for fish meal (Cho and Kim, 2010). Depending on the species of fish used and the manufacturing process, the nutritional value can vary between sources (Kim and Easter, 2001). Therefore, the manufacturing process and species of fish utilized to produce fish meal could be contributing to inconsistencies associated with fish meal in weaned pig diets (Jones et al., 2018).

Besides the species of fish, quality of fish meal can vary depending on freshness of the raw material, and processing method (Pike et al., 1990). Fish meal is produced by directly cooking or drying the raw fish and then extracting the oil (Cho and Kim, 2010). Fish meal can also vary in the amount of fish solubles added back from the manufacturing process. Fish solubles are a by-product of fish meal and oil production derived from the intermediate fraction of these products (Soares et al., 1973). In aquaculture, fish solubles have been used as a palatability enhancer but its effect on swine diets is still relatively unknown. On average fish meal can range from 8 to 15% fish solubles (Hertrampf and Piedad-Pascual).

Fermented or enzymatically treated soybean meal has recently been used to replace fish meal to lower diet cost. However, a higher-quality fish meal or fish solubles produced from whole fish rather than fish by-products may improve pig performance more than other protein sources, but data is not available to confirm this hypothesis. Therefore, the objective of these studies was to evaluate the influence of anchovy fish meal and fish solubles produced from whole fish compared to other common specialty protein sources on nursery pig growth performance, fecal dry matter, fecal consistency, morbidity and mortality.

Materials and Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments. Protein source nutrient, AA, and standardized ileal digestible (SID) values used in diet formulation were provided by manufacturers. Protein sources included: enzymatically treated soybean meal (HP 300; Hamlet Protein; Findlay, OH), Spray dried bovine plasma (APC Inc.; Ankeny, IA), Fermented soybean meal (MEPro; Priarie Aquatech; Brookings, SD), fish meal (TASA Prime meal; TASA; Lima, Peru), and fish solubles (TASA; Lima, Peru).

Ingredient and diet sampling and analysis. To confirm formulated values, each protein source from each experiment were submitted to the Agricultural Experiment Station Chemical Laboratory (University of Missouri-Columbia, Columbia, MO) for total AA analysis (method 982.30; AOAC International, 2006), crude protein (CP; method 990.03; AOAC International, 1990), dry matter (DM; method 935.29; AOAC International, 1990), ether extract (EE; ANKOM Technology, 2004), crude fiber (ANKOM Technology, 2005), and ash (method 942.05; AOAC International, 1990; Tables 1 and 2). Analysis of fermented soybean meal with solubles and fish meal with solubles was calculated using the chemical analysis of the amount of fermented soybean meal or fish meal and fish solubles. A sample of fish meal and fish solubles from each experiment were submitted to the New Jersey Feed Laboratory, Inc. (Trenton, NJ) for analysis of total volatile nitrogen (TVN; method 971.09; AOAC International, 2006) and biogenic amines (method by CSL Food Science Lab, Torry, Aberdeen Scotland; Table 3). Diet samples for Exp. 1 were collected from every fifth bag of manufactured feed using a feed probe to obtain a representative sample for each diet and phase. Diet samples from Exp. 2 were collected from each feeder using a feed probe to obtain a representative sample for each diet and phase. Diet samples were stored at -20°C until they were homogenized, subsampled, and submitted to the Agricultural Experiment Station Chemical Laboratories (University of Missouri-Columbia, Columbia, MO) for analysis of CP (method 990.03; AOAC International, 1990), DM (method 935.29; AOAC International, 1990), and EE (ANKOM Technology, 2004).

Experiment 1

Experiment 1 was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. This experiment utilized a single nursery room that was completely enclosed, environmentally controlled and mechanically ventilated. Each pen contained a 4-hole, dry self-feeder and a nipple waterer to provide *ad libitum* access to feed and water. Pens (1.3 × 1.3 m) had metal tri-bar floors and allowed approximately 0.42 m^2/pig or 0.34 m^2/pig for pens containing 4 and 5 pigs, respectively.

Animals and treatment structure. A total of 330 pigs (DNA 241 × 600; initially 4.9 ± 0.17 kg BW) were used in a 40-d nursery trial. Pigs were weaned at approximately 19-d of age and placed in pens of 4 or 5 pigs based on initial weight and gender. Each pen contained at least 2 barrows and 2 gilts. Due to a limited number of pigs, 42 pens were allotted with 5 pigs per pen (7 replications per treatment) and the remaining 30 pens were allotted with 4 pigs per pen (5 replications per treatment).

At weaning, pens were randomly allotted to 1 of 6 dietary treatments with 12 replications per treatment. Six dietary treatments were arranged in a one-way treatment structure with diets containing different protein sources. Protein sources added based on relative SID Lys content included enzymatically treated soybean meal (HP 300; Hamlet Protein, Findlay, OH) at 7.00% of the diet, spray-dried bovine plasma (APC Inc, Ankeny, IA) at 3.50% of the diet, fermented soybean meal (MEPro; Prairie Aquatech, Brooking, SD) at 5.00% of the diet, fermented soybean meal (MEPro; Prairie Aquatech, Brooking, SD) enriched with 6.5% fish solubles (TASA, Lima, Peru) at 5.21% of the diet, fish meal (TASA Prime meal; TASA, Lima, Peru) at 4.85% of the diet, or fish meal enriched with 6.5% fish solubles (TASA Swine, TASA, Lima, Peru) at 5.05% of the diet. Diets were formulated with similar levels of soybean meal and L-Lys HCl across all treatments. Individual pig weights and feed disappearance were measured on d 12, 19, 25, 33, and 40 to determine average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed-ratio (G:F).

Because of a delay in arrival of the fish meal source, all pigs were placed on a common phase 1 diet for 3 d after weaning. On d 3, all feeders were weighed, emptied, and refilled with experimental diets. On average, each pig consumed 0.2 kg of common phase 1 diet. Experimental phase 1 diets were fed until d 12 and phase 2 diets fed from d 12 to 25. Following phase 2, all pigs were fed a common diet for an additional 15 d (d 25 to 40). Diets (Tables 4 and 5) were formulated to contain 1.40% (phase 1) and 1.35% (phase 2) SID Lys and met or exceeded nutrient requirement estimates established by the National Research Council (NRC, 2012). The basal diets for phases 1 and 2 were manufactured at Hubbard Feeds in Beloit, KS. The basal diets were divided into 6 batches and protein sources were added and mixed at the

Kansas State O.H. Kruse Feed Technology Center in Manhattan, KS to form six experimental diets. Phase 1 and 2 diets were manufactured in pellet and meal form, respectively.

Fecal dry matter. Fecal samples were collected on d 12 and 25 to determine fecal DM percentage from the same three medium weight pigs from each pen. After collection, fecal samples were dried at 55°C in a forced air oven for 48 hours and the ratio of dried to wet fecal weight determined the fecal DM.

Experiment 2

Experiment 2 was conducted at a commercial research site owned and operated by New Horizon Farms in Pipestone, MN. This experiment utilized two identical nursery rooms that were completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contained a 6-hole, dry self-feeder and a pan waterer to provide *ad libitum* access to feed and water. Feed additions were accomplished using a robotic feeding system (FeedPro, FeedLogic Corp., Wilmar, MN). Pens (3.7 × 2.4 m) had plastic slatted floors and allowed approximately 0.36 m^2/pig or 0.33 m^2/pig for pens containing 25 and 27 pigs, respectively.

Animal and treatment structure. A total of 2,172 pigs (PIC L337 × 1050; initially 5.2 ± 0.62 kg BW) were used in a 42-d nursery trial across two rooms. The rooms were filled over the course of 7-d and all pens were balanced equally with gilts and barrows. Pigs were weaned at approximately 21-d of age and placed in pens of 25 or 27 pigs each based on initial weight and assigned to 1 of 6 dietary treatments with 14 replications per treatment in a randomized complete block design with pens blocked by body weight (BW) and weaning date.

The dietary treatment structure was the same as used in Exp. 1. Pens of pigs were weighed and feed disappearance was recorded on d 7, 14, 21, 28, 35, and 42 to determine ADG, ADFI, and G:F. Pigs were fed phase 1 diets from placement until d 7 and then offered phase 2 diets from d 7 to 21. Following phase 2, all pigs were placed on a common diet for an additional 21 d (d 21 to 42). Diets (Tables 6 and 7) were formulated to contain 1.40% (phase 1) and 1.35% (phase 2) SID Lys and met or exceeded nutrient requirement estimates established by the National Research Council (NRC, 2012). Phase 1 diets were manufactured in pellet form at Hubbard Feeds (Mankato, MN), while phase 2 diets were manufactured in meal form at New Horizon Farms (Pipestone, MN). Morbidity and mortality of treatments were also tracked because of a PRRS health challenge that occurred during the study. Pigs removed from pens because of morbidity were placed in a separate pen for cull pigs based on dietary treatment. Pigs placed in these pens were gruel fed initially and fed the same treatment diet as their original pen until the end of the study. Inventory, feed intake, and weight was collected at the end of the study to calculate treatment BW, ADG, ADFI, and G:F per pig placed on a closeout basis. Any mortalities within the cull pen were recorded as removed mortalities that was added to the total mortality percentage of the treatment.

Statistical analysis

In Exp. 1, data were analyzed as a completely randomized design using the RStudio environment (Version 1.3.1093, RStudio, Inc., Boston, MA) using R programming language [Version 4.0.2 (2020-06-22), R Core Team, R Foundation for Statistical Computing, Vienna, Austria] with pen as the experimental unit. Main effects of protein source were tested using the lm function of RStudio. Fecal dry matter was analyzed using the fixed effects of day, treatment, and the associated interaction accounting for repeated measures over time.

In Exp. 2, data were analyzed as a randomized complete block design for a one-way ANOVA using the RStudio environment (Version 1.3.1093, RStudio, Inc., Boston, MA) using R programming language [Version 4.0.2 (2020-06-22), R Core Team, R Foundation for Statistical Computing, Vienna, Austria] with pen serving as the experimental unit, weaning date and initial BW as a blocking factor, and treatment as fixed effect using the lmer function. Differences between treatments were determined using estimated marginal means. 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. Differences between treatments and day as well as their interactions were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results

Chemical analysis

Formulated values of protein sources from each experiment were confirmed for AA, DM, CP, EE, Crude fiber and ash based on laboratory analysis. Fish meal was found to contain 112 and 128 mg/kg of TVN for Exp. 1 and 2, respectively. Fish meal samples were also found to contain 132 and 609 mg/kg of histamine for Exp. 1 and 2, respectively. Fish solubles were found to contain 265 and 219 mg/kg of TVN for Exp. 1 and 2, respectively. Fish soluble samples were also found to contain 495 and 2,690 mg/kg of histamine for Exp. 1 and 2, respectively.

Experiment 1

Growth performance. In all weigh periods and overall, there were no differences for BW, ADG, ADFI, and G:F between treatments ($P > 0.10$). Although there was a 5 to 7% improvement in ADG during the experimental period for pigs fed spray-dried bovine plasma and fish meal with solubles compared to enzymatically treated soybean meal, the differences were not significant.

Fecal dry matter. There was a treatment × day interaction ($P = 0.003$) observed for fecal DM mainly driven by the large improvement in dry matter for pigs fed the diet with spray-dried bovine plasma from d 12 to 25. On d 12 (end of phase 1), there were no differences between treatments for fecal DM ($P > 0.05$). On d 25 (end of phase 2), pigs fed spray-dried bovine plasma had increased ($P = 0.017$) fecal DM compared to pigs fed enzymatically treated soybean meal, fermented soybean meal, fermented soybean meal with solubles, and fish meal with fish meal with solubles intermediate.

Experiment 2

In phase 1 (d 0 to 7), there were no differences between treatments for ADG, ADFI, and G:F ($P > 0.10$). In phase 2 (d 7 to 21), pigs fed fish meal had increased ($P < 0.05$) ADG compared to pigs fed fish meal with solubles with the other treatments intermediate. Pigs fed fermented soybean meal and fish meal had increased ($P < 0.05$) ADFI compared to pigs fed fish meal with solubles with other treatments intermediate. In the experimental period (d 0 to 21), pigs fed spray-dried bovine plasma and fish meal had increased ($P < 0.05$) ADG compared to pigs fed fish meal with solubles with the other treatments intermediate. Pigs fed fermented soybean meal and fish meal had increased ($P < 0.05$) ADFI compared to pigs fed fish meal with solubles with other treatments intermediate. Pigs fed spray-dried bovine plasma had increased ($P < 0.05$) G:F compared to pigs fed fish meal with solubles with the other treatments intermediate.

In the common period (d 21 to 42), there were no differences between treatments for ADG, ADFI, and G:F ($P > 0.10$).

Overall (d 0 to 42), there were no differences between treatments for ADFI and G:F ($P > 0.10$). Pigs fed fish meal tended to have numerically greater ($0.05 < P \leq 0.10$) ADG compared to other treatments, without significant mean separation. Pigs fed fish meal with solubles from d 0 to 21 had the highest ($P < 0.05$) mortality percentage compared to pigs fed spray-dried bovine plasma, fermented soybean meal with solubles, or fish meal with other treatments intermediate.

Discussion

Early nursery diets typically contain highly palatable and nutrient dense protein sources to encourage feed intake. Fish meal is considered a high-quality protein source because it contains AA that are generally deficient in cereal grains as well as vitamins and minerals that are often deficient in other protein sources (Church and Kellems, 1998). The response to fish meal is often inconsistent and could be reflection of factors associated with fish meal such as species of fish, processing method, and the amount of fish solubles added back to the meal. However, pigs fed a high-quality fish meal produced from whole fish rather than fish by-products may be a source of this inconsistency.

These experiments aimed to compare Peruvian anchovy fish meal and fish solubles produced from whole fish with various other specialty protein sources common in nursery pig diets. The additional protein sources utilized included enzymatically treated soybean meal, spray-dried bovine plasma, and fermented soybean meal. Enzymatically treated soybean meal is produced by treating soybean meal with microbial proteases and carbohydrases to reduce the levels of antinutritional factors and improve digestibility of amino acids and crude protein (Cervantes-Pahm and Stein; Goebel and Stein, 2011; Ma et al., 2019b). Fermented soybean meal is produced using a proprietary fermentation process to enhance protein and amino acid digestibility and reduce antinutritional factors present in conventional soybean meal (Jones et al., 2010). In recent years, fermented or enzymatically treated soybean meal has been used as a replacement to fish meal to lower diet cost. However, utilizing a high-quality fish meal may improve pig performance to provide an advantage compared with fermented or enzymatically treated soybean meal. Spray-dried bovine plasma is obtained from industrial fractionation of abattoir blood collection from healthy cattle and is spray dried to enhance bioactivity of proteins (Almeida et al., 2013; Crenshaw et al., 2017). Spray-dried animal plasma has been shown to increase growth performance in early nursery pigs (Pierce et al., 2005; Tran et al., 2014). A fish meal source that shows comparable results with spray-dried bovine plasma would provide justification for its inclusion in early nursery diets.

Fish meal destined for swine diets can be produced from a variety of fish species as well as from a wide range of geographical locations. Chile and Peru are producers of fish meal mainly consisting of Peruvian anchovies, Chilean jack mackerels, and Chub mackerels. However, in the United States, the majority of fish meal is produced from either Atlantic or Gulf Menhaden fish, while a lower percentage comes from Atlantic or Pacific herring and California sardines (Péron et al., 2010). Stoner et al. (1990) reported an improvement in growth performance from the addition of 4 to 8% menhaden fish meal. However, Jones et al. (2010) reported 3% menhaden fish meal was optimal to improve ADG and ADFI. In contrast, the current study utilized Peruvian anchovies as the fish meal source in weaned pig diets. Fish meal analysis reported by Li and Wu (2020) found anchovy fish meal to have a higher content of crude protein and total amino acids compared to menhaden fish meal making it a potentially higher quality fish meal source. However, Jones et al. (2018) reported no differences in growth performance between pigs fed anchovy fish meal or menhaden fish meal but observed an improvement from feeding increasing levels of anchovy fish meal from 0 to 6% of the diet. In contrast, the results of Exp. 1 in the current study observed no differences between pigs fed anchovy fish meal or other specialty protein sources. However, in Exp. 2 results observed pigs fed fish meal tended to have greater ADG compared to enzymatically without significant mean separation in the overall data.

An additional factor that differs between fish meal sources is the amount of fish solubles added back during the manufacturing process. Fish meal commonly contains between 8 to 15% fish solubles (Jones et al., 2018). Fish solubles contain high amounts of the water-soluble nitrogen compounds such as proteins, peptides, amino acids, and nucleotides (Oterhals and Samuelsen, 2015). In addition, various soluble and insoluble fractions of B-vitamins and minerals are also found in fish solubles (Soares et al., 1973). Therefore, additional fish solubles in nursery pig diets may further improve growth performance.

In aquaculture, fish solubles have been used as a protein source and palatability enhancer. Utilizing fish solubles in aquaculture diets supported growth and feed efficiency (Kousoulaki et al., 2009; Zhang et al., 2021). Fish solubles was found to be a negligible protein source in poultry diets, but when fed in combination with fish meal it was able to improve feed intake and subsequent growth compared to fish meal alone (Laksesvela, 1958; Hulan and Proudfoot, 1987). Therefore, intake may also be enhanced by the addition of fish solubles in swine diets. Jones et al. (2018) reported no differences with increased fish soluble fractions; however, the authors did report an improvement in growth performance from diets containing fish meal with solubles compared a control diet. The current study utilized anchovy fish solubles whereas Jones et al. (2018) utilized menhaden fish solubles. Additionally, there is little research on the effects of fish solubles in combination with a specialty vegetable protein source such as fermented soybean meal. To our knowledge, the current study is the first to determine the influence of fish solubles in combination with fermented soybean meal on nursery pig performance. In Exp. 1 pigs fed fish meal with solubles did have a 5% improvement in ADG compared to enzymatically treated soybean meal, although results were not significant. There were no differences when comparing pigs fed fish meal or fish meal with solubles, similar to the results reported by Jones et al.

(2018). Fermented soybean meal with solubles also showed no differences compared to fermented soybean meal alone. In contrast, in Exp. 2, pigs fed fish meal with solubles had decreased ADG and ADFI in the experimental period compared to fish meal without added solubles. Interestingly, the addition of fish solubles to fermented soybean meal did not decrease performance as it did when added to fish meal.

Freshness and quality of fish meal and fish solubles may be a contributor to inconsistencies among studies. Traditionally, freshness and quality measures of fish meal are determined by total volatile nitrogen (TVN) and biogenic amines. Total volatile nitrogen is a determination of the protein quality within fish meal, which measures the amount of free N as an indication of the protein degradation (Kjelden et al., 1983). Fish soluble and fish meal samples from both experiments were submitted for analysis of TVN. A value less than 0.15% is generally an indication of good quality (Jones et al., 2018). The fish meal samples from Exp. 1 and 2 were found to contain 0.112 and 0.128% TVN, respectively. The fish soluble samples from Exp. 1 and 2 were found to contain 0.219 and 0.265% TVN, respectively. The TVN values could explain why solubles present in fermented soybean meal and fish meal treatments did not improve performance. Biogenic amines are an indication of AA bioavailability in fish products (Jasour et al., 2018). According to FAO (2018), a histamine level of 1,000 mg/kg or above indicates poor quality, while a histamine level of 500 mg/kg indicates high quality. Fish meal samples were found to contain 132 and 609 mg/kg for Exp. 1 and 2, respectively. Fish solubles utilized in Exp. 1 were found to contain 495 mg/kg of histamine indicating good AA bioavailability whereas the fish solubles in Exp. 2 were found to contain 2,690 mg/kg of histamine which could have contributed to the poor performance of pigs fed fish meal with solubles in this experiment.

Fecal DM of anchovy fish meal and fish solubles were also compared to those of other protein sources in Exp. 1. Fecal DM for pigs fed anchovy fish meal with and without solubles was comparable to those fed other protein sources, with the exception of spray-dried bovine plasma on d 25. The interaction observed for fecal DM was mainly driven by the large improvement in fecal DM for pigs fed spray-dried bovine plasma from d 12 to 25. As a result, pigs fed spray-dried bovine plasma had increased fecal DM percentage on d 25 compared to those fed other protein sources. Ultimately, anchovy fish meal or fish solubles does not influence fecal dry matter observations.

Pig removals and mortality in response to dietary treatment were also analyzed in Exp. 2. The research site experienced a significant PRRS health challenge during the study which led to high instances of morbidity and mortality. Pigs fed fish meal with solubles had higher mortality percentage compared with pigs fed spray-dried bovine plasma, fermented soybean meal with fish solubles, or fish meal. Reasons for the increase in mortality of pigs fed fish meal with solubles remains unclear because pigs fed fermented soybean meal with solubles or fish meal did not experience high mortality.

In conclusion, the results of Exp. 1 indicate anchovy fish meal produces comparable results with other protein sources for nursery pig growth and fecal consistency. Fish meal with solubles, although not significant, numerically increased ADG along with spray-dried bovine plasma. In contrast, the results of Exp. 2 indicate anchovy fish meal with solubles decreased growth performance compared to other protein sources. Reasons for decreased growth remain unclear, but may be associated with the higher histamine and TVN content of the fish solubles used in Exp. 2. Fish meal without added solubles was able to improve gain during the experimental period and fermented soybean meal with fish solubles was comparable with fermented soybean meal alone. Ultimately, both experiments indicate fish meal without added solubles can be fed to nursery without impacting growth performance but added fish solubles in combination with fish meal requires further investigation on its impact in nursery pig diets.

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Table 3.1 Chemical and calculated analysis of protein sources, Exp. 1[1]

Item, %	Enzymatically treated SBM	Bovine plasma	Fermented SBM	Fermented SBM with solubles[2]	Fish meal	Fish meal with solubles[3]
DM	92.45	88.68	94.53	92.08	92.43	90.11
CP	55.63	76.21	72.39	70.24	66.73	64.94
EE	2.12	0.00	1.61	1.80	8.76	8.48
Crude fiber	4.08	0.00	6.69	6.26	0.28	0.26
Ash	6.86	8.04	1.33	2.16	16.75	16.58
Amino acids						
Ala	2.37	3.70	3.08	3.02	4.14	4.01
Arg	3.84	4.39	5.09	4.86	3.76	3.62
Asp	6.16	7.74	8.38	7.97	5.91	5.66
Cys	0.81	2.57	1.03	0.97	0.65	0.61
Glu	9.90	10.53	13.20	12.61	8.44	8.16
Gly	2.30	2.68	3.07	3.06	4.09	4.01
His	1.40	2.29	1.85	1.87	2.25	2.25
Ile	2.69	2.46	3.74	3.54	2.86	2.72
Leu	4.20	7.13	5.86	5.59	4.78	4.58
Lys	3.14	6.86	4.36	4.22	5.28	5.08
Met	0.76	0.93	0.99	0.96	1.77	1.69
Phe	2.87	4.05	4.03	3.81	2.71	2.58
Pro	2.72	3.97	3.80	3.64	2.65	2.57
Ser	2.36	4.42	3.27	3.11	2.21	2.12
Thr	2.12	5.01	2.77	2.65	2.71	2.59
Trp	0.71	1.44	1.02	0.96	0.73	0.69
Tyr	2.04	3.90	2.69	2.54	2.08	1.97
Val	2.74	5.35	3.76	3.58	3.29	3.14

[1] A sample of each protein source was collected, homogenized, subsampled, and submitted to the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO) for proximate analysis and amino acid profile.

[2] Analysis of fermented soybean meal with solubles was calculated using the chemical analysis of fermented soybean meal (ME-PRO; Priarie Aquatech; Brookings, SD) and fish solubles (TASA; Lima, Peru).

[3] Analysis of fish meal with solubles (TASA Swine; TASA, Lima, Peru) was calculated using the chemical analysis of fish meal (TASA Prime meal; TASA, Lima, Peru) and fish solubles (TASA, Lima, Peru).

Table 3.2 Chemical and calculated analysis of protein sources, Exp. 2[1]

Item, %	Enzymatically treated SBM	Bovine plasma	Fermented SBM	Fermented SBM with solubles[2]	Fish meal	Fish meal with solubes[3]
DM	93.44	90.51	95.46	93.06	93.06	90.66
CP	54.04	77.43	70.96	69.18	66.32	64.74
EE	0.57	0.00	0.00	0.07	9.55	8.96
Crude fiber	4.75	0.00	6.75	6.31	0.85	0.79
Ash	7.16	8.31	2.15	2.89	16.18	15.99
Amino acids						
Ala	2.30	3.78	3.19	3.13	3.96	3.85
Arg	3.74	4.47	4.77	4.58	3.70	3.57
Asp	5.91	7.77	7.94	7.58	5.82	5.58
Cys	0.77	2.56	1.02	0.96	0.66	0.62
Glu	9.40	10.59	11.79	11.33	8.02	7.79
Gly	2.34	2.74	3.12	3.11	3.78	3.72
His	1.41	2.38	1.81	1.85	5.16	2.17
Ile	2.76	2.56	3.92	3.72	2.97	2.83
Leu	4.17	7.37	5.97	5.71	4.72	4.53
Lys	3.23	6.97	3.97	3.88	5.16	4.98
Met	0.73	0.93	1.06	1.03	1.73	1.65
Phe	2.85	4.21	3.96	3.75	2.70	2.57
Pro	2.81	4.26	3.88	3.73	2.64	2.56
Ser	2.07	3.97	2.80	2.68	1.99	1.91
Thr	1.97	4.73	2.63	2.52	2.55	2.44
Trp	0.67	1.43	0.83	0.79	0.71	0.67
Tyr	1.93	3.85	2.66	2.52	2.03	1.93
Val	2.81	5.60	3.98	3.80	3.41	3.25

[1] A sample of each protein source was collected, homogenized, subsampled, and submitted to the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO) for proximate analysis and amino acid profile.

[2] Analysis of fermented soybean meal with solubles was calculated using the chemical analysis of fermented soybean meal (ME-PRO; Priarie Aquatech; Brookings, SD) and fish solubles (TASA; Lima, Peru).

[3] Analysis of Fish meal with solubles (TASA Swine; TASA, Lima, Peru) was calculated using the chemical analysis of fish meal (TASA Prime meal; TASA, Lima, Peru) and fish solubles (TASA, Lima, Peru).

Table 3.3 Total volatile nitrogen and biogenic amine analysis of fish meal and fish solubles

Item	Total volatile nitrogen (mg/100g)	Histamine (mg/kg)
Fish meal[2]		
Exp. 1	112	132
Exp. 2	128	609
Fish solubles[2]		
Exp. 1	265	495
Exp. 2	219	2,690

[1] A sample of fish meal and fish solubles was collected and submitted to the New Jersey Feed Laboratory Inc. (Ewing, NJ) for analysis of total volatile nitrogen and biogenic amines.

[2] TASA, Lima, Peru

Table 3.4 Phase 1 diet composition (as-fed basis), Exp. 1[1]

Item	Enzymatically treated SBM[2]	Bovine plasma[2]	Fermented SBM[2]	Fermented SBM with solubles[2]	Fish meal[2]	Fish meal with solubles[2]
Ingredients, %						
Corn	37.89	41.67	39.78	39.57	40.77	40.54
Soybean meal (46.5% CP)	23.72	23.72	23.72	23.72	23.72	23.72
Whey powder	25.00	25.00	25.00	25.00	25.00	25.00
Enzymatically treated soybean meal	7.00	---	---	---	---	---
Fermented soybean meal	---	---	5.00	---	---	---
Fermented soybean meal with solubles	---	---	---	5.21	---	---
Fish meal	---	---	---	---	4.85	---
Fish meal with solubles	---	---	---	---	---	5.05
Spray-dried bovine plasma	---	3.50	---	---	---	---
Corn oil	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	0.81	0.83	0.76	0.76	0.51	0.53
Monocalcium phosphate (21% P)	0.65	0.55	0.78	0.78	0.18	0.20
Salt	0.33	0.13	0.35	0.35	0.30	0.30
L-Lys-HCl	0.35	0.35	0.35	0.35	0.35	0.35
DL-Met	0.23	0.21	0.23	0.23	0.21	0.21
L-Thr	0.15	0.15	0.15	0.15	0.18	0.18
L-Trp	0.02	0.02	0.02	0.02	0.02	0.02

L-Val	0.08	0.10	0.08	0.08	0.13	0.13
Zinc oxide	0.39	0.39	0.39	0.39	0.39	0.39
Vitamin premix[3]	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
Total	100	100	100	100	100	100

Table 3.4. (cont.)

SID amino acids, %						
Lys	1.40	1.40	1.40	1.40	1.40	1.40
Ile:Lys	62	56	62	61	58	58
Leu:Lys	116	114	119	118	111	111
Met:Lys	36	34	36	36	38	38
Met and Cys:Lys	58	58	58	58	58	58
Thr:Lys	64	64	64	64	64	64
Trp:Lys	19.5	19.3	19.3	19.2	19.3	19.3
Val:Lys	70	70	70	70	70	70
His:Lys	35	35	36	36	35	35
Total Lys, %	1.53	1.54	1.54	1.54	1.55	1.54
NE NRC, kcal/kg	2,615	2,628	2,619	2,619	2,632	2,632
SID Lys:NE, g/Mcal	5.36	5.33	5.35	5.35	5.32	5.32
CP, %	21.9	21.0	21.8	21.8	21.6	21.6
Ca, %	0.76	0.74	0.75	0.75	0.75	0.75
P, %	0.64	0.61	0.63	0.63	0.63	0.63
STTD P, %	0.54	0.54	0.54	0.54	0.54	0.54
Analyzed values, %						
DM	91.32	91.38	91.66	91.30	91.09	90.87

CP	18.73	18.83	19.29	19.79	19.31	19.18
Ether extract	3.66	3.84	3.83	3.62	3.80	3.72
Crude fiber	2.08	1.85	2.13	2.23	1.67	1.81
Ash	7.37	6.62	6.38	7.69	6.30	6.32

[1] Phase 1 diets were fed from approximately 5.0 to 5.9 kg.

[2] 1) HP 300; Hamlet Protein, Findlay, OH. 2) Spray-dried bovine plasma; APC Corp, Ankeny, IA. 3) ME-PRO; Prairie Aquatech, Brookings, SD. 4) ME-PRO with fish solubles; Prairie Aquatech, Brookings, SD; TASA, Lima, Peru. 5) TASA Prime meal; TASA, Lima, Peru. 6) TASA Swine; TASA, Lima, Peru.

[3] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.13% STTD P with 1,250 FTU/kg.

Table 3.5 Phase 2 diet composition (as-fed basis), Exp. 1[1]

Item	Enzymatically treated SBM[2]	Bovine plasma[2]	Fermented SBM[2]	Fermented SBM with solubles[2]	Fish meal[2]	Fish meal with solubles[2]
Ingredients, %						
Corn	53.37	57.15	55.27	55.07	56.28	56.02
Soybean meal (46.5% CP)	24.82	24.82	24.82	24.82	24.82	24.82
Whey powder	10.00	10.00	10.00	10.00	10.00	10.00
Enzymatically treated soybean meal	7.00	---	---	---	---	---
Fermented soybean meal	---	---	5.00	---	---	---
Fermented soybean meal with solubles	---	---	---	5.21	---	---
Fish meal	---	---	---	---	4.85	---
Fish meal with solubles	---	---	---	---	---	5.05
Spray-dried bovine plasma	---	3.50	---	---	---	---
Corn oil	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	0.91	0.93	0.88	0.86	0.61	0.63
Monocalcium phosphate (21% P)	0.90	0.80	1.03	1.03	0.40	0.45
Salt	0.55	0.35	0.55	0.55	0.53	0.53
L-Lys-HCl	0.38	0.38	0.38	0.38	0.38	0.38
DL-Met	0.20	0.18	0.20	0.20	0.19	0.19
L-Thr	0.15	0.15	0.15	0.15	0.18	0.18

L-Trp	0.02	0.02	0.03	0.03	0.03	0.03
L-Val	0.06	0.09	0.07	0.07	0.11	0.11
Zinc oxide	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[3]	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
Total	100	100	100	100	100	100

Table 3.5. (cont.)

SID amino acids, %						
Lys	1.35	1.35	1.35	1.35	1.35	1.35
Ile:Lys	62	55	61	61	58	58
Leu:Lys	120	119	123	123	116	115
Met:Lys	36	33	36	36	38	38
Met and Cys:Lys	58	58	58	58	58	58
Thr:Lys	63	63	63	63	63	63
Trp:Lys	19.4	19.3	19.2	19.2	19.3	19.2
Val:Lys	70	70	70	70	70	70
His:Lys	37	37	38	38	38	38
Total Lys, %	1.49	1.50	1.50	1.50	1.50	1.50
NE NRC, kcal/kg	2,493	2,507	2,498	2,498	2,513	2,513
SID Lys:NE, g/Mcal	5.41	5.39	5.40	5.40	5.37	5.38
CP, %	22.0	21.1	21.9	21.9	21.7	21.7
Ca, %	0.76	0.73	0.76	0.75	0.74	0.75
P, %	0.63	0.61	0.63	0.63	0.62	0.63
STTD P, %	0.51	0.51	0.51	0.51	0.51	0.51
Analyzed values, %						
DM	90.06	89.98	90.23	90.21	90.25	90.09

CP	22.84	17.08	18.75	18.41	17.58	18.47
Ether extract	2.43	2.27	2.39	2.36	2.94	2.85
Crude fiber	2.13	2.06	2.29	2.32	1.74	1.83
Ash	6.12	5.73	5.50	5.92	5.93	6.69

[1] Phase 2 diets were fed from approximately 5.9 to 11.8 kg.

[2] 1) HP 300; Hamlet Protein, Findlay, OH. 2) Spray-dried bovine plasma; APC Corp, Ankeny, IA. 3) ME-PRO; Prairie Aquatech, Brookings, SD. 4) ME-PRO with fish solubles; Prairie Aquatech, Brookings, SD; TASA, Lima, Peru. 5) TASA Prime meal; TASA, Lima, Peru. 6) TASA Swine; TASA, Lima, Peru.

[3] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.13% STTD P with 1,250 FTU/kg.

Table 3.6 Phase 1 diet composition (as-fed basis), Exp. 2[1]

Item	Enzymatically treated SBM[2]	Bovine plasma[2]	Fermented SBM[2]	Fermented SBM with solubles[2]	Fish meal[2]	Fish meal with solubles[2]
Ingredients, %						
Corn	38.11	41.89	40.01	39.80	41.00	40.76
Soybean meal (46.5% CP)	23.70	23.70	23.70	23.70	23.70	23.70
Whey powder	25.00	25.00	25.00	25.00	25.00	25.00
Enzymatically treated soybean meal	7.00	---	---	---	---	---
Fermented soybean meal	---	---	5.00	---	---	---
Fermented soybean meal with solubles	---	---	---	5.21	---	---
Fish meal	---	---	---	---	4.85	---
Fish meal with solubles	---	---	---	---	---	5.05
Spray-dried bovine plasma	---	3.50	---	---	---	---
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	0.81	0.83	0.76	0.76	0.51	0.53
Monocalcium phosphate (21% P)	0.65	0.55	0.78	0.78	0.18	0.20
Salt	0.33	0.13	0.35	0.35	0.30	0.30
L-Lys-HCl	0.35	0.35	0.35	0.35	0.35	0.35
DL-Met	0.23	0.21	0.23	0.23	0.21	0.21
L-Thr	0.15	0.15	0.15	0.15	0.18	0.18

L-Trp	0.02	0.02	0.02	0.02	0.02	0.02
L-Val	0.08	0.10	0.08	0.08	0.13	0.13
Zinc oxide	0.39	0.39	0.39	0.39	0.39	0.39
Vitamin premix	0.05	0.05	0.05	0.05	0.05	0.05
Trace mineral premix	0.08	0.08	0.08	0.08	0.08	0.08
Inorganic selenium	0.05	0.05	0.05	0.05	0.05	0.05
Phytase[3]	0.02	0.02	0.02	0.02	0.02	0.02
Total	100	100	100	100	100	100

Table 3.6. (cont.)

SID amino acids, %						
Lys	1.40	1.40	1.40	1.40	1.40	1.40
Ile:Lys	62	56	62	61	58	58
Leu:Lys	116	114	119	118	111	111
Met:Lys	36	34	36	36	38	38
Met and Cys:Lys	58	58	58	58	58	58
Thr:Lys	64	64	64	64	64	64
Trp:Lys	19.5	19.3	19.3	19.2	19.3	19.3
Val:Lys	70	70	70	70	70	70
His:Lys	35	35	36	36	35	35
Total Lys, %	1.53	1.54	1.54	1.54	1.55	1.54
NE NRC, kcal/kg	2,615	2,628	2,619	2,619	2,632	2,632
SID Lys:NE, g/Mcal	5.36	5.33	5.35	5.35	5.32	5.32
CP, %	21.9	21.0	21.8	21.8	21.6	21.6
Ca, %	0.76	0.74	0.75	0.75	0.75	0.75
P, %	0.64	0.61	0.63	0.63	0.63	0.63
STTD P, %	0.53	0.53	0.53	0.53	0.53	0.53
Analyzed values, %						
DM	88.63	90.67	89.68	89.83	89.19	88.95

CP	19.86	19.82	20.13	19.70	19.90	20.52
Ether extract	3.79	3.79	3.95	4.00	4.05	4.11
Crude fiber	2.15	2.15	1.62	1.72	1.53	1.62
Ash	6.36	6.37	6.38	6.27	5.94	5.98

[1] Phase 1 diets were fed from approximately 5.1 to 5.7 kg.

[2] 1) HP 300; Hamlet Protein, Findlay, OH. 2) Spray-dried bovine plasma; APC Corp, Ankeny, IA. 3) ME-PRO; Prairie Aquatech, Brookings, SD. 4) ME-PRO with fish solubles; Prairie Aquatech, Brookings, SD; TASA, Lima, Peru. 5) TASA Prime meal; TASA, Lima, Peru. 6) TASA Swine; TASA, Lima, Peru.

[3] Quantum Blue 5G (ABVista; Plantation, FL) provided an estimated release of 0.12% STTD P with 751 FTU/kg.

Table 3.7 Phase 2 diet composition (as-fed basis), Exp. 2[1]

Item	Enzymatically treated SBM[2]	Bovine plasma[2]	Fermented SBM[2]	Fermented SBM with solubles[2]	Fish meal[2]	Fish meal with solubles[2]
Ingredients, %						
Corn	53.54	57.32	55.39	55.18	56.41	56.18
Soybean meal (46.5% CP)	24.75	24.75	24.75	24.75	24.75	24.75
Whey powder	10.00	10.00	10.00	10.00	10.00	10.00
Enzymatically treated soybean meal	7.00	---	---	---	---	---
Fermented soybean meal	---	---	5.00	---	---	---
Fermented soybean meal with solubles	---	---	---	5.21	---	---
Fish meal	---	---	---	---	4.85	---
Fish meal with solubles	---	---	---	---	---	5.05
Spray-dried bovine plasma	---	3.50	---	---	---	---
Choice white grease	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	0.91	0.93	0.88	0.86	0.61	0.63
Monocalcium phosphate (21% P)	0.90	0.80	1.03	1.03	0.40	0.45
Salt	0.55	0.35	0.55	0.55	0.53	0.53
L-Lys-HCl	0.38	0.38	0.38	0.38	0.38	0.38
DL-Met	0.20	0.18	0.20	0.20	0.19	0.19
L-Thr	0.19	0.19	0.19	0.19	0.22	0.22

L-Trp	0.02	0.02	0.03	0.03	0.03	0.03
L-Val	0.06	0.09	0.07	0.07	0.11	0.11
Zinc oxide	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin-trace mineral premix	0.20	0.20	0.20	0.20	0.20	0.20
Phytase[3]	0.06	0.06	0.06	0.06	0.06	0.06
Total	100	100	100	100	100	100

Table 3.7. (cont.)

SID amino acids, %						
Lys	1.35	1.35	1.35	1.35	1.35	1.35
Ile:Lys	62	55	61	61	58	58
Leu:Lys	120	119	123	123	116	115
Met:Lys	36	33	36	36	38	38
Met and Cys:Lys	58	58	58	58	58	58
Thr:Lys	63	63	63	63	63	63
Trp:Lys	19.4	19.3	19.2	19.2	19.3	19.2
Val:Lys	70	70	70	70	70	70
His:Lys	37	37	38	38	38	38
Total Lys, %	1.49	1.50	1.50	1.50	1.50	1.50
NE NRC, kcal/kg	2,493	2,507	2,498	2,498	2,513	2,513
SID Lys:NE, g/Mcal	5.41	5.39	5.40	5.40	5.37	5.38
CP, %	22.0	21.1	21.9	21.9	21.7	21.7
Ca, %	0.76	0.73	0.76	0.75	0.74	0.75
P, %	0.63	0.61	0.63	0.63	0.62	0.63
STTD P, %	0.51	0.51	0.51	0.51	0.51	0.51
Analyzed values, %						
DM	88.02	88.03	88.84	88.61	88.31	88.29

CP	21.01	22.37	22.12	22.38	20.25	20.59
Ether extract	1.97	2.00	1.95	2.00	3.48	3.61
Crude fiber	1.97	1.66	2.01	2.02	2.11	1.75
Ash	6.01	5.61	5.83	5.54	6.06	5.93

[1] Phase 2 diets were fed from approximately 5.7 to 9.4 kg.

[2] 1) HP 300; Hamlet Protein, Findlay, OH. 2) Spray-dried bovine plasma; APC Corp, Ankeny, IA. 3) ME-PRO; Prairie Aquatech, Brookings, SD. 4) ME-PRO with fish solubles; Prairie Aquatech, Brookings, SD; TASA, Lima, Peru. 5) TASA Prime meal; TASA, Lima, Peru. 6) TASA Swine; TASA, Lima, Peru.

[3] Optiphos 2000 (Huvepharma; Peachtree City, GA) provided an estimated release of 0.12% STTD P with 1,201 FTU/kg.

Table 3.8 Effects of protein source on nursery pig performance and fecal dry matter, Exp. 1[1,2]

Protein source:	Enzymatically treated SBM[3]	Bovine plasma[3]	Fermented SBM[3]	Fermented SBM with solubles[3]	Fish meal[3]	Fish meal with solubles[3]	SEM	*P* =
BW, kg								
d 0	4.9	4.9	4.8	4.9	4.9	4.9	0.05	0.996
d 12	5.9	6.1	5.9	6.0	6.1	6.1	0.11	0.507
d 25	11.4	11.9	11.5	11.5	11.7	11.9	0.26	0.652
d 40	19.8	20.5	19.8	19.8	20.1	20.3	0.42	0.731
Day 0 to 12[4]								
ADG, g	87	101	84	88	96	103	8.1	0.447
ADFI, g	161	153	139	143	153	164	8.7	0.283
G:F	0.54	0.66	0.61	0.60	0.63	0.62	0.034	0.241
Day 12 to 25								
ADG, g	422	450	432	425	429	446	13.6	0.618
ADFI, g	574	618	592	569	573	609	18.1	0.278
G:F	0.74	0.73	0.73	0.75	0.75	0.73	0.009	0.387
Day 0 to 25 (Experimental period)								
ADG, g	261	282	265	263	268	281	9.4	0.426
ADFI, g	375	395	374	365	370	396	11.8	0.318
G:F	0.69	0.72	0.71	0.72	0.73	0.71	0.010	0.339

Day 25 to 40 (Common period)								
ADG, g	557	573	554	548	558	562	15.8	0.926
ADFI, g	901	887	890	889	877	879	26.0	0.990
G:F	0.62	0.65	0.62	0.62	0.64	0.64	0.010	0.231
Day 0 to 40 (Overall)								
ADG, g	372	390	373	370	376	386	9.9	0.619
ADFI, g	573	578	568	561	560	577	13.6	0.901
G:F	0.65	0.68	0.66	0.66	0.67	0.67	0.008	0.206
Fecal DM, %[5]								
d 12	19.6	17.0	18.7	19.1	19.5	19.1	0.89	0.330
d 25	19.0[b]	22.3[a]	19.3[b]	19.2[b]	19.3[b]	21.1[ab]	0.67	0.003

[1] A total of 330 pigs (initial BW of 4.9 ± 0.17 kg) were used in a 40-d nursery trial with 4 or 5 pigs per pen and 12 pens per treatment. Pigs were weaned at approximately 19-d of age and allotted to treatment in a completely randomized design. Dietary treatments were arranged in a one-way treatment structure with main effects of protein source.

[2] Pens of pigs were fed diets in 2 phases. Pigs were fed phase 1 diets from 0 to 12 d after weaning. Following phase 1, pigs were fed phase 2 diets from d 12 to 25. Following the experimental period, all pigs were fed a common diet from d 25 to 40.

[3] 1) HP 300; Hamlet Protein, Findlay, OH. 2) Spray-dried bovine plasma; APC Corp, Ankeny, IA. 3) ME-PRO; Prairie Aquatech, Brookings, SD. 4) ME-PRO with fish solubles; Prairie Aquatech, Brookings, SD; TASA, Lima, Peru. 5) TASA Prime meal; TASA, Lima, Peru. 6) TASA Swine; TASA, Lima, Peru.

[4] All pigs were placed on a common phase 1 diet for 3 d after weaning. Once experimental diets arrived, all feeders were weighed, dumped, and refilled with experimental diets. On average, each pig consumed 0.2 kg of the common phase 1 diet.

[5] Treatment × day, *P* = 0.003; Treatment, *P* = 0.782; Day, *P* = 0.009. The *P*-values represented in the data table show the effect of treatment within day. Fecal dry matter superscripts compare treatments as well as day.

Table 3.9 Effects of protein source on nursery pig performance in a commercial environment, Exp. 2[1,2]

Protein source:	Enzymatically treated SBM[3]	Bovine plasma[3]	Fermented SBM[3]	Fermented SBM with solubles[3]	Fish meal[3]	Fish meal with solubles[3]	SEM	*P* =
BW, kg								
d 0	5.2	5.2	5.2	5.2	5.2	5.2	0.23	0.984
d 7	5.8	5.9	5.8	5.8	5.8	5.8	0.22	0.936
d 21	9.7	9.9	9.9	9.9	10.0	9.4	0.36	0.132
d 42	19.3	19.5	19.4	19.8	20.0	19.2	0.67	0.469
d 42 closeout[4]	19.0	19.0	19.0	19.3	19.6	19.2	---	---
Day 0 to 7								
ADG, g	85	96	83	87	85	77	14.5	0.397
ADFI, g	219	219	213	209	214	209	9.3	0.510
G:F	0.36	0.42	0.38	0.41	0.39	0.36	0.066	0.486
Day 7 to 21								
ADG, g	253[ab]	266[ab]	271[ab]	267[ab]	279[a]	242[b]	12.6	0.016
ADFI, g	401[ab]	403[ab]	433[a]	421[ab]	427[a]	389[b]	12.9	0.004
G:F	0.63	0.66	0.62	0.63	0.65	0.62	0.018	0.289
Day 0 to 21 (Experimental period)								
ADG, g	190[ab]	204[a]	202[ab]	202[ab]	208[a]	182[b]	10.9	0.004
ADFI, g	334[ab]	336[ab]	353[a]	344[ab]	349[a]	324[b]	9.9	0.008

G:F	0.57[ab]	0.60[a]	0.57[ab]	0.58[ab]	0.59[ab]	0.56[b]	0.020	0.012
Day 21 to 42 (Common period)								
ADG, g	443	451	440	453	468	447	18.3	0.342
ADFI, g	662	665	656	670	689	646	25.5	0.342
G:F	0.67	0.68	0.67	0.67	0.68	0.69	0.008	0.275
Day 0 to 42 (Overall)								
ADG, g	308	322	314	320	330	306	12.0	0.061
ADFI, g	487	493	496	498	509	476	14.3	0.135
G:F	0.63	0.65	0.63	0.64	0.65	0.64	0.010	0.160
Day 0 to 42 (Overall) closeout[5]								
ADG, g	275	291	285	299	304	265	---	---
ADFI, g	470	472	475	486	493	446	---	---
G:F	0.59	0.62	0.60	0.62	0.62	0.59	---	---
Day 0 to 42 (Overall)								
Removals, %	13.2	9.2	11.2	12.2	11.2	8.9	3.23	0.324
Mortality, %	4.3[ab]	1.9[b]	2.9[ab]	2.3[b]	2.3[b]	7.2[a]	2.56	0.002
Total mortality, %[6]	11.9	8.3	9.9	8.0	8.6	14.9	---	---

[1] A total of 2,172 pigs (initial BW of 5.2 ± 0.62 kg) were used in a 42-d nursery trial with 25 or 27 pigs per pen and 14 pens per treatment spread across two rooms. Pigs were weaned at approximately 21-d of age and allotted to treatment in a randomized complete block design with weight and weaning data as the blocking factor. Dietary treatments were arranged in a one-way treatment structure with main effects of protein source.

[2] Pens of pigs were fed diets in 2 phases. Pigs were fed phase 1 diets from 0 to 7 d after weaning. Following phase 1, pigs were fed phase 2 diets from d 7 to 21. Following the experimental period, all pigs were fed a common diet from d 21 to 35.

[3] 1) HP 300; Hamlet Protein, Findlay, OH. 2) Spray-dried bovine plasma; APC Corp, Ankeny, IA. 3) ME-PRO; Prairie Aquatech, Brookings, SD. 4) ME-PRO with fish solubles; Prairie Aquatech, Brookings, SD; TASA, Lima, Peru. 5) TASA Prime meal; TASA, Lima, Peru. 6) TASA Swine; TASA, Lima, Peru.

[4] d 42 closeout = total ending weight / (number of pigs placed × days of trial). Includes removed pigs. Removed pigs were only followed according to treatment and not pen originally removed from; therefore, no SEM or *P* values are reported.

[5] Total treatment gain or intake / (number of pigs placed × days of trial). Includes removed pigs. Removed pigs were only followed according to treatment and not pen originally removed from; therefore, no SEM or *P* values are reported.

[6] Total mortality = mortalities + removed mortalities. Removed pigs were only followed according to treatment and not pen originally removed from; therefore, no SEM or *P* values are reported.