Evaluating the effects of specialty protein sources on nursery pig performance

by

Aaron Michael Jones

B.S., Purdue University, 2012
M.S., Purdue University, 2015

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry
College of Agriculture

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Manhattan, Kansas

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Abstract

A total of 6,465 nursery pigs were used in 8 experiments. Experiment 1 investigated the effects of *Lactobacillus plantarum* (LP) or fermented soybean meal (FSBM) on nursery pig growth performance. A LP × FSBM interaction was detected for G:F, where LP and FSBM individually improved G:F, but the effect was not additive. Experiment 2 evaluated the effects of increasing levels of LP on nursery pig performance. No evidence for differences in growth performance were observed among dietary treatments. Experiment 3 and 4 examined the effects of fish meal source and level on nursery pig growth performance. Overall, a source × level interaction for ADG, G:F and final BW was observed as increasing fish meal source 1 improved ADG and G:F; however, pigs fed fish meal source 2 had improved ADG and G:F at 3%, but decreased at 6%. Pigs fed fish meal source 3 had no further improvements in ADG and G:F beyond the 3% inclusion. No evidence for differences were detected between the dietary treatments for ADFI. Experiment 5 evaluated the effects of feeding fish solubles on nursery pig performance. Pigs fed diets with fish meal had increased ADG and ADFI compared to pigs fed the control diet. There was no evidence for differences in growth performance as fish solubles increased. Experiment 6 and 7 investigated the effects of enzymatically-treated soybean meal (ESBM) on nursery pig performance. Results indicated that nursery pigs fed diets with greater than 9% of ESBM resulted in decreased ADFI and final BW. Experiment 8 evaluated the effects of dietary electrolyte balance (dEB) on nursery pig performance. Increasing dEB in diets from weaning to 21-d after weaning resulted in an increase in ADG and BW, which was the result of a marginally significant improvement in ADFI and G:F. Finally, an experiment was conducted to determine the optimal strategy for collecting and submitting samples that adequately describe the nutrient levels in diets collected from a commercial swine facility. Sampling feeders with a probe

resulted in less variability on an individual basis, but seemed to get washed out when individual samples were pooled to form a composite sample.

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Dedication

This dissertation is dedicated to my family.

Chapter 1 - Evaluating the effects of *Lactobacillus plantarum* and fermented soybean meal on nursery pig performance

ABSTRACT

Two experiments with 360 weanling pigs each (Exp. 1 = 5.52 kg BW; Exp. 2 = 5.95 kg BW) were conducted to evaluate the effects of *Lactobacillus plantarum* (LP) or fermented soybean meal (FSBM) on nursery pig growth performance. In Exp. 1, pigs were allotted to pens based on initial BW in a completely randomized design. Dietary treatments were arranged in a 2 × 2 factorial with main effects of FSBM (0 vs. 8%) and LP (0 vs. 0.1%). There were 10 pigs per pen and 9 pens per treatment. Experimental diets were fed in two phases, d 0 to 14 and 14 to 24 with a common diet fed from d 24 to 45. During d 0 to 14, pigs fed diets containing FSBM had decreased ($P < 0.05$) ADG, ADFI, and d 14 BW compared with pigs fed diets without FSBM. No evidence for differences was detected among pigs fed diets with or without LP. From d 14 to 24, there was no evidence for differences observed among dietary treatments. From d 0 to 24, pigs fed diets containing FSBM tended to have decreased ($P = 0.088$) ADFI; however, no evidence of differences were observed for ADG, G:F, or d 24 BW. Pigs fed diets containing LP tended to have improved ($P = 0.053$) G:F compared with pigs fed diets without LP, with no evidence of differences observed for ADG, ADFI, or d 24 BW. During the common period (d 24 to 45), there was a tendency ($P = 0.093$) for a LP × FSBM interaction for G:F where pigs previously fed FSBM had improved G:F when fed alone, but no difference was observed for those previously fed FSBM and LP. For the overall nursery period (d 0 to 45), a LP × FSBM interaction was observed for G:F ($P = 0.021$) where LP and FSBM individually improved G:F, but the effect was not additive. In Exp. 2, pigs were allotted to pens based on initial weight in a completely randomized design to 1 of 4 dietary treatments with 10 pigs per pen and 9 pens per

treatment. Dietary treatments contained 0, 0.05, 0.1, and 0.2% LP. Experimental diets were fed in 3 phases: d 0 to 7, 7 to 21, and 21 to 42. During d 0 to 21, no evidence for differences were detected among the dietary treatments. From d 21 to 42, ADG and ADFI were not influenced by treatment; however, increasing LP marginally improved G:F (quadratic, $P = 0.085$). Overall, no evidence for differences in growth performance were observed among dietary treatments. In conclusion, these data suggest that the FSBM used in this study tended to result in decreased ADFI in nursery pigs immediately after weaning, with LP providing little to no benefit on performance.

Key words: fermented soybean meal, growth, *Lactobacillus plantarum, nursery pig*

INTRODUCTION

Feed intake in the pig is often low and variable directly after weaning. Thus, research has focused on stimulating feed intake to subsequently increase performance (Pluske et al., 1997). One option is to use highly palatable and nutrient dense protein sources in nursery diets to stimulate feed intake. Traditionally, this has been accomplished with the addition of milk- and animal-based ingredients. However, cost and bio-security concerns related to the transmission of porcine epidemic diarrhea virus (PEDv) in ingredients, such as spray-dried porcine plasma, has led many producers to seek other alternatives (Dee et al., 2014; Cochrane et al., 2015). One product that has gained interest is the use of fermented soybean meal (FSBM) which is derived from the fermentation of conventional soybean meal using a mixed culture of bacteria and fungus (Hong et al., 2004; Wang et al., 2014).

Likewise, the use of probiotics has been a focus within the swine industry in recent years as a potential antibiotic alternative. Among the diverse bacterial species used for probiotics, the nonpathogenic class of *bacillus* species are some of the most extensively studied. Of the species,

L. plantarum has shown some of the more promising beneficial results on the overall gastrointestinal microbiota of nursery pigs (Guerra-Ordaz et al., 2013). *Lactobacillus plantarum* is a facultative heterofermentative plant-associated lactic acid bacterium that is tolerant to bile salts and low pH, improving survivability in the gastrointestinal tract (Guerra-Ordaz et al., 2013; da Silva Sabo et al., 2014). However, these studies have been conducted under highly controlled environments with research examining its impact in commercial settings scarce. In addition, previous studies have shown that mixtures of multi-strain probiotics may be more effective than singles strains in the prevention of pathogen growth in the gastrointestinal tract (Chapman et al., 2012).

Therefore, the objectives of our research were to: 1) evaluate the growth performance of nursery pigs fed FSBM and LP independently and together, and 2) evaluate the efficacy of increasing LP fed to nursery pigs.

MATERIALS AND METHODS

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments. These experiments were conducted at a commercial research facility located in northeast Ohio. The facility is a totally enclosed, environmentally controlled, and mechanically ventilated building. Each pen (1.53 × 1.83 m) had slatted metal floors and was equipped with a 4-hole stainless steel feeder and one nipple-cup waterer for ad libitum access to feed and water. The facility was equipped with a computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN) that delivered and recorded daily feed additions and diets as specified. This system is capable of feeding each individual pen any of the individual diets. Diets were manufactured at a commercial feed mill (Kalmbach Feeds, Inc., Upper

Sandusky, OH) and delivered to individual bulk bins at the research site. Nursery rooms were not power washed or disinfected after the previous group of pigs to provide high levels of environmental bacteria load.

Experiment 1

A total of 360 pigs (C-29 × 359 PIC, Hendersonville, TN; initially 5.52 kg) were used in a 45-d study with 10 pigs per pen and 9 pens per treatment. Pigs were weaned at approximately 18 to 20 d of age and allotted to pens based on initial weight in a completely randomized design to 1 of 4 dietary treatments. Dietary treatments were arranged in a 2 × 2 factorial with main effects of FSBM (NF8; Nutraferma, Sioux City, IA; 0 vs. 8%) and LP (LactoPlan; Nutraferma, Sioux City, IA; 0 vs 0.1%). Pigs and feeders were weighed on d 0, 7, 14, 24, 35, and 45 of the trial to determine ADG, ADFI, and G:F.

Experimental diets were fed in two phases from d 0 to 14 and 14 to 24 (Table 1-1). A common diet was then fed to all pigs from d 24 to 45 post-weaning. Diets were formulated to contain 1.40, 1.35, and 1.30% standardized ileal digestible (SID) Lys from d 0 to 7, 7 to 24, and 24 to 45, respectively. Nutrient values and SID coefficients for the FSBM used in diet formulation were provided by the manufacturer (Nutraferma, Sioux City, IA). All diets were fed in pellet form.

Experiment 2

A total of 360 pigs (C-29 × 359, initially 5.95 kg) were used in a 42-d trial with 10 pigs per pen and 9 pens per treatment. Pigs were weaned at approximately 16 to 20 d of age and allotted to pens based on initial weight and gender to 1 of 4 dietary treatments in a completely randomized design. Experimental diets were fed in three phases, d 0 to 7, 7 to 21, and 21 to 42 post-weaning for phase 1, 2, and 3, respectively. Treatment diets were formulated to include 0,

0.05, 0.10, or 0.20% LP (LactoPlan; Nutraferma, Sioux City, IA). All treatment diets within phase were formulated to similar nutrient levels with LP added at the expense of corn (Table 1-2). Pigs and feeders were weighed every 7 d to determine ADG, ADFI, and G:F. All experimental diets were fed in pellet form.

Diet Sampling and Analysis

In Exp. 1 and 2, diet samples were obtained from feeders each week during the study, composited, and frozen at -20ºC for subsequent analysis. Samples of FSBM were collected at the feed mill during diet manufacturing during Exp. 1, composited, and frozen at -20ºC. Composite samples of diets and FSBM were split using a riffle splitter (Humboldt Mfg. Co., Norridge, IL) and processed through a 1 mm screen in a Willey mill (Thomas Scientific, Swedesboro, NJ) prior to analysis. All samples of diets and protein source were submitted to Ward Laboratories Inc. (Kearney, NE) for analysis of DM (method 935.29; AOAC International, 2012), CP (method 990.03; AOAC International., 2012), ether extract (method 920.39; AOAC International, 2012) for preparation and analyzed using an ANKOM XT20 Fat Analyzer (Ankom Technology, Fairport, NY), Ca and P (method 968.08; AOAC International, 2012) for preparation and analyzed using an ICAP 6500 (ThermoElectron Corp., Waltham, MA). The complete AA profile for the FSBM was analyzed (method 982.30; AOAC International, 2006) by the University of Missouri-Columbia College of Agriculture Experiment Station Chemical Laboratories (Columbia, MO).

Statistical Analysis

Data were analyzed using the PROC GLIMMIX procedure in SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit and dietary treatment as a fixed effect. For Exp. 1, the main effects and interactions of LP and FSBM were tested. Differences between treatments

were determined by using least square means. In Exp. 2, preplanned linear and quadratic polynomial contrasts were used to determine the effects of increasing LP on performance criteria. Results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

RESULTS

Chemical Analysis

Results from proximate analysis of experimental diets and the FSBM were similar to formulated values (Tables 1-3 to 1-5). The FSBM used in Exp. 1 contained less Lys, Leu, and Phe than formulated values. Whereas, all other AA values were similar to formulated values.

Growth Performance

Experiment 1.

During d 0 to 14, pigs fed diets containing FSBM had decreased ($P < 0.05$) ADG, ADFI, and d 14 BW compared with pigs fed diets without. No evidence for differences was detected among pigs fed diets with or without LP. From d 14 to 24, there was no evidence for differences observed among pigs fed any of the dietary treatments. From d 0 to 24, pigs fed diets containing FSBM tended to have decreased ($P = 0.088$) ADFI; however, no evidence of differences were observed for ADG, G:F, or d 24 BW. However, pigs fed diets containing LP tended to have improved ($P = 0.053$) G:F compared with pigs fed diets without LP, with no evidence of differences observed for ADG, ADFI, or d 24 BW. During d 24 to 45 (common period), there was a tendency ($P = 0.093$) for a LP × FSBM interaction for G:F where pigs previously fed FSBM had improved G:F, but the effect was not additive when both LP and FSBM were fed in combination. There was no evidence for differences observed for the main effects of LP or FSBM on ADG, ADFI, G:F, or d 45 BW. For the overall nursery period, a LP × FSBM

interaction was detected for G:F (P = 0.021) where LP and FSBM fed from d 0 to 24 individually improved G:F, but the diet containing both additives was intermediate.

Experiment 2.

During d 0 to 7 and 7 to 21, no evidence for differences was detected with increasing LP. From d 21 to 42, ADG and ADFI were not influenced by treatment; however, increasing LP marginally improved G:F (quadratic, P = 0.085). For the overall nursery period, no evidence for differences in ADG, ADFI, G:F, or d 42 BW were observed among dietary treatments.

DISCUSSION

Fermented soybean meal is a product derived from the fermentation of conventional soybean meal using a mixed starter culture of bacteria or fungus (Hong et al., 2004). The principle function of the fermentation process is to hydrolyze the soy proteins into small peptides, thus significantly reducing the total concentrations of anti-nutritional factors that may be found in conventional soybean meal with the intent of improving nutrient availability (Hong et al., 2004; Mukherjee et al., 2016). While *Bacillus* species have been traditionally used in the production of FSBM, often a combination of bacterial species are utilized to improve the nutritional and functional properties of FSBM (Mukherjee et al., 2016). The FSBM product used in Exp. 1 was manufactured from conventional soybean meal and utilized a patented proprietary blend of lactic acid bacteria *Pedicoccus pentosaceus* and *bacillus subtilis*.

Much of the published literature investigating FSBM has shown beneficial results. Min et al. (2004) conducted a study evaluating the replacement of conventional SBM with increasing FSBM (0, 2.5, 5.0, and 7.5%) containing *Aspergillus oryza*e and *Bacillus subtilis*. In their study, increasing FSBM resulted in a linear increase in ADG and ADFI in weanling pigs. However, Jones et al. (2010) observed only an improvement in G:F when a similar FSBM product

containing *Aspergillus oryza*e and *Bacillus subtilis* was fed to weanling pigs. These results are consistent with the findings of Cho et al. (2008) and Kim et al. (2010), which also observed improvements in G:F, but not ADG when weanling pigs were fed a FSBM product inoculated with *Aspergillus oryza*e GB-107.

Interestingly, in the study herein, when FSBM was added at 8% of the diet and fed for 24 d, ADFI was marginally reduced, with no evidence for differences detected for ADG and G:F. These results are contrary to the previous research reported above. The reasons for the different responses observed are unclear. However, a key contrast between our study and previous studies is that the bacterial mixtures, doses, and methodologies used in the preparation of the FSBM differed. In addition, the difference in responses observed by Min et al. (2004) and our results to FSBM could be in relation to the amount of SBM that FSBM replaced. Friesen et al. (1993) reported linear reductions in growth performance when early-weaned pigs were fed diets with increasing SBM. Experimental diets fed by Min et al. (2004) had amounts of SBM ranging from 39.4% (Negative control diet; 0% FSBM) to 30.2% (7.5% FSBM). Thus, it might be expected that replacing conventional SBM with up to 7.5% FSBM would elicit an improvement in growth based on the inclusion rates of conventional SBM used. Another possible explanation for the decreased performance for pigs fed FSBM could be contributed to the lower analyzed Lys content relative to the nutrient value used in diet formulation (2.99 vs. 3.20). However, based on the relatively low inclusion of FSBM, this would not have had a major impact on total diet Lys concentrations.

The marginal reduction in feed intake observed in our study with the FSBM is not completely understood. However, sensory tests with humans has demonstrated that soy products that have been modified by enzymes are often perceived as being bitter and producing an

astringent taste (Cho et al., 2004). The formation of the bitterness is believed to be the result of the proteolytic enzymes exposing the hydrophobic AA found in the interior portion of the protein, resulting in the hydrophobic portion of protein interacting with the taste buds to produce a bitter taste (Kurst et al., 2003). This is particularly relevant in that soy peptides are degraded during the fermentation process by microbial proteolytic enzymes (Hong et al., 2004). However, more research is needed to determine if this effect is preference related.

It is generally believed that the oral supplementation of bacteria enhanced products containing probiotic mixtures may have beneficial effects against a wide range of pathogenic bacteria known to cause enteric disease (Chapman et al., 2012). This may be due to strain-specific effects of individual species used in the mixture, or simply a greater total concentration of bacteria provided in a multi-strain preparation (Chapman et al., 2012). With this in mind, we wanted to determine whether the combination of the FSBM product containing *Pedicoccus pentosaceus* and *bacillus subtilis* in combination with *Lactobacillus plantarum* would provide a beneficial additive or synergistic effect.

Like the other species of bacteria found in the FSBM, *Lactobacillus plantarum* a facultative gram positive lactic acid bacteria. Of the vast array of bacterial species, *L. plantarum* has shown some of the more promising beneficial results on the overall gastrointestinal microbiota of nursery pigs (Guerra-Ordaz et al., 2013). Previous research has also indicated that probiotics may increase nutrient digestibility and improve growth performance in nursery pigs (Miguel et al., 2004; Lee et al., 2012; Cai et al., 2015). However, most studies that have evaluated the efficacy of probiotics has been conducted under highly controlled environments with research examining its impact in commercial settings being scarce.

In our studies, an inconsistent response was observed, in which pigs fed 0.10% LP in Exp. 1 had a marginal improvement in G:F, but when pigs were fed increasing levels of LP (0 – 0.2%) in Exp. 2 no growth benefit was observed for pigs fed LP. Previous studies suggest inconsistency in growth responses when feeding probiotics to pigs are not uncommon (Keegan et al., 2005; LeJeune et al., 2006). Jacela et al. (2010) suggested that production practices, dose of probiotic, and health status are all factors that may contribute to inconsistency in growth responses from one study to another. In the present studies, nursery rooms were not power washed or disinfected after the previous group of pigs to provide higher levels of environmental bacteria load. However, based on the minimal number of pigs removed from the study (Exp. 1 = 3 pigs removed; Exp. 2 = 3 pigs removed) and overall growth performance, health was not an issue with the group of pigs used in our studies. Furthermore, a marked difference between our study and previous studies (Miguel et al., 2004; Lee et al., 2012; Cai et al., 2015) that have found a response is that the individual species or combination of species used were different. Collado et al. (2007) indicated significant variability between strains of individual probiotics and combinations of probiotics in their efficacy for combating certain pathogens. Interestingly, the combination of FSBM and LP provided no improvement in ADG, ADFI, or G:F in Exp. 1.

In conclusion, our data suggest that inclusion of this particular FSBM in nursery pig diets tended to result in poorer ADFI immediately after weaning, with LP providing very little to no benefit on performance. Furthermore, the combination of FSBM and LP provided no additive or synergistic effect when fed together.

LITERATURE CITED

AOAC International. 2006. Official Methods of Analysis of AOAC Int. 18th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

AOAC International. 2012. Official Methods of Analysis of AOAC Int. 19th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

Cai, L., S. Indrakumar, E. Kiarie, and I. H. Kim. 2015. Effects of a multi-strain *Bacillus* species-based direct-fed microbial on growth performance, nutrient digestibility, blood profile, and gut health in nursery pigs fed corn-soybean meal-based diets. J. Anim. Sci. 93:4336-4342. doi: 10.2527/jas2015-9056.

Chapman, C. M. C., G. R. Gibson, and I. Rowland. 2012. In vitro evaluation of single-and-multi-strain probiotics: Inter-species between probiotic strains, and inhibition of pathogens. J. Anaerobe 18:405-413. doi:10.1016/j.anaerobe.2012.05.004.

Cho, J. H., B. J. Min, Y. J. Chen, J. S. Yoo, Q. Wang, J. H. Ahn, I. B. Chung, and I. H. Kim. 2008. Evaluation of FSP (fermented soy protein) to replace soybean meal in weaned pigs: Growth performance, blood urea nitrogen and total protein concentrations in serum and nutrient digestibility. J. Anim. Sci. 86 (Suppl. 3):95 (Abstr.).

Cho, M. J., N. Unklesbay, F. Hsih, A. D. Clark. 2004. Hydrophobicity of bitter peptides from soy protein hydrolysates. J. Agric. Food Chem. 52:5895-5901. doi:10.1021/jf0495035.

Cochrane, R. A., S. S. Dritz, J. C. Woodworth, C. R. Stark, A. R. Huss, J. P. Cano, R. W. Thompson, A. C. Fahrenholz, and C. K. Jones. 2015. Feed mill biosecurity plans: a systematic approach to prevent biological pathogens in swine feed. J. Swine Health Prod. 3:154-164.

Collado, M. C., J. Meriluoto, and S. Salminen. 2007. Role of commercial probiotic strains against human pathogen adhesion to intestinal mucus. Letters Appl. Microbiol. 45:454-460. doi:10.1111/j.1472-765X.2007.02212.x.

da Silva Sabo, S., M. Vitolo, J. M. Domínguez González, and R. P. d. S. Oliveira. 2014. Overview of *Lactobacillus plantarum* as a promising bacteriocin producer among lactic acid bacteria. Food Res. Int. 64:527-536. doi: 10.1016/j.foodres.2014.07.041.

Dee, S., T. Clement, A. Schelkopf, J. Nerem, D. Knudsen, J. C. Hennings, and E. Nelson. 2014. An evaluation of contaminated complete feed as a vehicle for porcine epidemic diarrhea virus infection of naïve pigs following consumption via natural feeding behavior: proof of concept. BMC Vet Res. 10:176. doi: 10.1186/s12917-04-0176-9.

Friesen, K. G., R. D. Goodband, J. L. Nelssen, F. Blecha, D. N. Reddy, P. G. Reddy, and L. J. Kats. 1993. Effect of pre- and postweaning exposure to soybean meal on growth performance and on the immune response in the early-weaned pig. J. Anim. Sci. 71:2089-2098. doi: 10.2527/1993.7182089x.

Guerra-Ordaz, A. A., G. Gonzalez-Ortiz, R. M. La Ragione, M. J. Woodward, J. W. Collins, J. F. Perez, S. M. Martin-Orue. 2013. Effect of inclusion of lactulose and *Lactobacillus plantarum* on the intestinal environment and performance of piglets at weaning. Anim. Feed Sci. Tech. 185: 160-168. doi: 10.1016/j.anifeedsci.2013.07.009.

Hong, K. J., C. H. Lee, and S. W. Kim. 2004. Aspergillus oryzae GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. J. Med. Food 7:430–434. doi:10.1089/jmf.2004.7.430.

Jacela, J. Y., J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. L. Nelssen, D. G. Renter, and S. S. Dritz. 2010. Feed additives for swine: Fact sheets- prebiotics and probiotics, and phytogenics. J. Swine Health Prod. 3:132-136.

Jones, C. K., J. M. DeRouchey, J. L. Nelssen, M. D. Tokach, S. S. Dritz, and R. D. Goodband. 2010. Effects of fermented soybean meal and specialty animal protein sources on nursery pig performance. J. Anim. Sci. 88:1725-1732. Doi: 10.2572/jas.2009-2010.

Keegan, T. P., S. S. Dritz, J. L. Nelssen, J. M. DeRouchey, M. D. Tokach, and R. D. Goodband. 2005. Effects of in-feed antimicrobial alternatives and antimicrobials on nursery pig performance and weight variation. J. Swine Health Prod. 1:12-18.

Kim, S. W., E. van Heugten, F. Ji, C. H. Lee, and R. D. Mateo. 2010. Fermented soybean meal as a vegetable protein source for nursery pigs: I. Effects on growth performance of nursery pigs. J. Anim. Sci. 88:214-224. doi: 10.2527/jas.2009-1993.

Kurst, T. 2003. Protein modification to optimize functionality: Protein hydrolysates. In: R. Whitaker, A. G. J. Voragen, D. W. S. Wong, editors. Handbook of food enzymology. New York: Marcel Dekker, Inc. p. 221-253.

Lee, J. S., E. G. Awji, S. J. Lee, D. D. Tassew, Y. B. Park, K. S. Park, M. K. Kim, B. Kim, and S. C. Park. 2012. Effect of *Lactobacillus plantarum* CJLP243 on the growth performance and cytokine response of weanling pigs challenged with enterotoxigenic *Escherichia coli.* J. Anim. Sci. 90:3709-3717. doi: 10.2527/jas2011-4434.

LeJeune, J. T., M. D. Kauffman, M. D. Amstutz, and L. A. Ward. 2006. Limited effects of a commercial direct-fed microbial on weaning pig performance and gastrointestinal microbiology. J. Swine Health Prod. 5:247-252.

Miguel, J. C., S. L. Rodrigques-Zas, and J. E. Pettigrew. 2004. Efficacy of a mannan oligosaccharide (Bio-Mos) for improving nursery pig performance. J. Swine Health Prod. 12:296-307.

Min, B. J., J. W. Hong, O. S. Kwon, W. B. Lee, Y. C. Kim, I. H. Kim, W. T. Cho, and J. H. Kim. 2004. The effect of feeding processed soy protein on the growth performance and apparent illeal digestibility in weanling pigs. Asian-Australas. J. Anim. Sci. 17:1271-1726. doi: 10.5713/ajas.2004.1271.

Mukherjee, R., R. Chakraborty, and A. Dutta. 2016. Role of fermentation in improving nutritional quality of soybean meal – a review. Asian-Australas. J. Anim. Sci. 11:1523-1529. doi: 10.5713/ajas.15.0627.

Pluske, J. R., D. J. Hampson, and I. H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livest. Prod. Sci. 51:215-236. doi: 10.1016/S0301-6226(97)00057-2.

Wang, Y., X.T. Liu, H.L. Wang, D.F. Li, X.S. Piao, and W.Q. Lu. 2014. Optimization of processing conditions for solid-state fermented soybean meal and its effects on growth performance and nutrient digestibility of weanling pigs. Livest. Sci. 170:91-99. doi: 10.1016/j.livsci.2014.07.02.

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

	d 0 to 14		d 14 to 24		d 24 to 45
	Control	FSBM	Control	FSBM	Common
Ingredient, %					
Corn	28.00	28.59	38.09	38.61	52.02
Soybean meal, 46.5% CP	35.03	26.50	36.00	27.50	32.50
Corn DDGS	10.00	10.00	10.00	10.00	10.00
Spray dried whey	21.75	21.75	10.85	10.85	---
FSBM[3]	---	8.00	---	8.00	---
Tallow	2.00	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.05	1.10	1.15
Moncalcium P, 21% P	0.85	0.75	0.75	0.65	1.10
Salt	0.25	0.25	0.30	0.30	0.40
L-Lys HCl	0.24	0.28	0.23	0.27	0.37
DL-Met	0.15	0.15	0.12	0.12	0.14
L-Thr	0.09	0.09	0.09	0.09	0.15
L-Trp	---	---	---	---	0.01
Phytase[4]	0.01	0.01	0.01	0.01	0.01
Zinc oxide	0.40	0.40	0.26	0.26	---
Choline chloride, 70%	0.04	0.04	0.04	0.04	---
Trace mineral premix[5]	0.11	0.11	0.11	0.11	0.11
Vitamin premix[6]	0.10	0.10	0.10	0.10	0.05
TOTAL	100	100	100	100	100
Calculated analysis					
Standardized ileal digestible (SID) amino acids, %					
Lys	1.40	1.40	1.35	1.35	1.30
Met:Lys	34	35	33	33	35
Met & Cys:Lys	58	58	58	58	58
Thr:Lys	65	65	65	65	65
Trp:Lys	20	20	20	20	18
Val:Lys	71	71	73	73	69
ME, kcal/kg	3,351	3,358	3,353	3,360	3,340
CP, %	24.55	24.80	24.57	24.83	22.91
Ca, %	0.96	0.96	0.90	0.90	0.92
P, %	0.86	0.84	0.80	0.80	0.81
Available P, %	0.59	0.59	0.50	0.50	0.50

[1]*Lactobacillus plantarum* (LactoPlan; Nutraferma, Sioux City, IA) was included in the diet at 0.10% at the expense of corn for those treatments that contained it.

[2]Dried distillers grains with solubles.

[3]Fermented soybean meal (NF8; Nutraferma, Sioux City, IA).

[4]Quantum Blue (AB-Vista Americas, Plantation, FL) provided 500 phytase units (FTU)/kg of diet, with a release of 0.13% available P.

[5]Provided per kg of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulfate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[6]Provided per kg of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; 15 mg vitamin B12.

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

Ingredient, %	d 0 to 7	d 7 to 21	d 21 to 42
Corn	35.67	41.45	52.01
Soybean meal, 46.5% CP	30.00	30.00	32.54
Corn DDGS[1]	5.00	10.00	10.00
Spray dried whey	21.74	10.87	---
Fish meal	2.50	3.00	---
Tallow	2.00	2.00	2.00
Limestone	1.06	0.93	1.13
Monocalcium P, 21% P	0.80	0.40	1.09
Sodium chloride	0.25	0.30	0.40
L-Lys HCl	0.22	0.28	0.37
DL-Met	0.15	0.12	0.14
L-Thr	0.09	0.11	0.15
L-Trp	0.01	0.02	0.01
Phytase[2]	0.01	0.01	0.01
Zinc oxide	0.26	0.26	---
Choline chloride, 70%	0.04	0.04	---
Selenium premix, 0.06%	0.02	0.02	0.02
Trace mineral premix[3]	0.09	0.09	0.09
Vitamin premix[4]	0.10	0.10	0.05
LP[5]	---	---	---
TOTAL	100	100	100
Calculated analysis			
Standardized ileal digestible (SID) amino acids, %			
Lys	1.40	1.35	1.30
Met:Lys	33	35	35
Met & Cys:Lys	58	58	58
Thr:Lys	65	65	65
Trp:Lys	20	20	18
Val:Lys	70	71	69
ME, kcal/kg	3,375	3,378	3,342
CP, %	23.36	23.92	22.92
Ca, %	0.96	0.91	0.91
P, %	0.85	0.78	0.81
Available P, %	0.59	0.50	0.50

[1]Dried distillers grains with solubles.

[2]Quantum Blue (AB-Vista Americas, Plantation, FL) provided 500 phytase units (FTU)/kg of diet, with a release of 0.13% available P.

[3]Provided per kg of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulfate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[4]Provided per kg of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; 15 mg vitamin B12.

[5]*Lactobacillus Plantarum* (LactoPlan; Nutraferma Inc., Sioux City, IA) was substituted at 0.05, 0.10, or 0.20% of the diet at the expense of corn to form the treatments.

Table 1-3. Chemical analysis of diets, Exp. 1[1]

	Phase 1				Phase 2				Phase 3
Item, %	Control	LP[2]	FSBM[3]	LP + FSBM	Control	LP[2]	FSBM[3]	LP + FSBM	Common diet
DM	89.87	90.34	91.86	91.52	90.07	90.39	89.70	90.73	88.40
CP	24.2	24.6	23.2	24.4	23.4	24.2	24.8	24.5	22.9
Ether extract	4.2	4.0	4.1	3.9	4.5	4.7	4.6	4.6	4.9
Ca	0.93	0.93	1.02	0.88	0.87	0.86	0.81	0.83	0.92
P	0.72	0.75	0.65	0.65	0.57	0.66	0.67	0.58	0.70

[1]Complete diet samples were obtained from each dietary treatment each week during the study and composited. Samples of the diets were then submitted to Ward Laboratories, Inc. (Kearny, NE) for analysis.

[2]*Lactobacillus plantarum* (LactoPlan; Nutraferma, Sioux City, IA).

[3]Fermented soybean meal (NF8; Nutraferma, Sioux City, IA).

Table 1-4. Chemical analysis of fermented soybean meal (FSBM), Exp. 1

Item, %[2]	FSBM[1]
DM	94.56
CP	51.56
Ether extract	1.5
Ca	0.60
P	0.77
Total AA, %[3]	
Arg	3.65 (3.70)
Cys	0.73 (0.77)
His	1.36 (1.37)
Ile	2.49 (2.21)
Leu	3.96 (4.25)
Lys	2.99 (3.20)
Met	0.73 (0.71)
Phe	2.58 (2.87)
Thr	1.99 (2.02)
Trp	0.75 (0.65)
Tyr	1.73 (2.08)
Val	2.65 (2.32)

[1]Fermented soybean meal (NF8; Nutraferma, Sioux City, IA).

[2]Proximate analysis for FSBM was analyzed by Ward Laboratories Inc., (Kearney, NE).

[3]Amino acid analysis for FSBM was analyzed by the University of Missouri-Columbia College of Agriculture, Food and Natural Resources – Agriculture Experiment Station Chemical Laboratories (Columbia, MO). Values in parentheses indicate values used in diet formulation.

Table 1-5. Chemical analysis of diets, Exp. 2[1]

Item, %	Control	LP[2]		
		0.05%	0.10%	0.20%
Phase 1 diets				
DM	89.68	90.20	90.40	90.66
CP	22.4	22.1	23.2	21.2
Ether extract	4.9	4.7	4.8	4.7
Ca	0.80	0.84	0.75	0.77
P	0.71	0.72	0.70	0.66
Phase 2 diets				
DM	89.87	89.09	88.95	89.53
CP	22.40	21.30	22.40	23.10
Ether extract	5.6	5.3	5.7	5.7
Ca	0.75	0.77	0.78	0.75
P	0.66	0.66	0.64	0.63
Phase 3 diets				
DM	88.35	88.27	88.75	89.16
CP	20.	22.1	22.8	22.5
Ether extract	5.3	5.2	5.4	5.2
Ca	0.73	0.74	0.72	0.71
P	0.67	0.67	0.68	0.70

[1]Complete diet samples were obtained from each treatment each week during the study and composited. Samples of diets were then submitted for analysis by Ward Laboratories, Inc. (Kearney, NE).

[2]*Lactobacillus plantarum* (LactoPlan; Nutraferma, Sioux City, IA.

Table 1-6. Effects of Lactobacillus plantarum and fermented soybean meal on nursery pig performance, Exp. 1[1]

						Probability, *P* <		
	Control	LP[2]	FSBM[3]	LP + FSBM	SEM	LP × FSBM	LP	FSBM
BW, kg								
d 0	5.52	5.51	5.52	5.53	0.012	0.301	0.892	0.392
d 14	7.62	7.66	7.48	7.49	0.076	0.816	0.737	0.045
d 24	10.86	11.04	10.84	10.98	0.136	0.890	0.247	0.751
d 45	22.78	23.42	23.07	23.29	0.276	0.459	0.130	0.782
d 0 to 14								
ADG, g	150	154	140	138	5.4	0.588	0.837	0.026
ADFI, g	169	160	155	153	4.1	0.480	0.172	0.013
G:F	0.887	0.961	0.901	0.904	0.0260	0.181	0.148	0.429
d 14 to 24								
ADG, g	324	338	336	349	10.4	0.980	0.199	0.270
ADFI, g	492	491	478	489	9.8	0.566	0.631	0.454
G:F	0.655	0.684	0.701	0.711	0.0190	0.539	0.287	0.166
d 0 to 24								
ADG, g	222	231	222	226	5.8	0.709	0.294	0.663
ADFI, g	303	298	290	292	5.4	0.545	0.789	0.088
G:F	0.734	0.774	0.765	0.773	0.0122	0.210	0.053	0.244
Common diet (d 24 to 45)								
ADG, g	568	589	582	586	9.4	0.346	0.183	0.551
ADFI, g	811	836	812	840	13.8	0.934	0.065	0.862
G:F	0.700	0.705	0.718	0.698	0.0070	0.093	0.319	0.453
d 0 to 45								
ADG, g	383	398	390	393	6.2	0.334	0.142	0.832
ADFI, g	539	549	534	547	8.5	0.881	0.178	0.658
G:F	0.710[a]	0.723[b]	0.732[b]	0.721[ab]	0.0050	0.021	0.826	0.055

[ab] Means within the same row with different superscripts differ ($P < 0.05$).

[1]A total of 360 pigs (PIC C-29 × 359) with 10 pigs per pen and 9 replications per treatment were used in a 45-d growth trial.

[2]*Lactobacillus plantarum* (LactoPlan) and fermented soybean meal (NF8) (Nutraferma, Sioux City, IA) were fed from d 0 to 24.

Table 1-7. Effect of increasing *Lactobacillus plantarum* on nursery pig performance, Exp. 2[1]

		LP[2]				Probability, $P <$	
Diets	Control	0.05%	0.10%	0.20%	SEM	Linear	Quadratic
BW, kg							
d 0	5.95	5.94	5.94	5.95	0.008	0.616	0.455
d 7	6.42	6.36	6.37	6.40	0.048	0.962	0.394
d 21	11.17	10.92	10.91	11.02	0.137	0.601	0.178
d 42	23.53	23.34	23.16	23.17	0.297	0.402	0.612
d 0 to 7							
ADG, g	67	60	61	64	6.4	0.894	0.456
ADFI, g	104	100	98	103	4.3	0.955	0.321
G:F	0.644	0.578	0.606	0.614	0.0472	0.846	0.477
d 7 to 21							
ADG, g	339	325	324	330	8.5	0.606	0.209
ADFI, g	407	398	387	393	8.9	0.276	0.260
G:F	0.833	0.816	0.839	0.842	0.0115	0.326	0.651
d 21 to 42							
ADG, g	588	589	584	579	9.4	0.405	0.919
ADFI, g	821	810	802	807	13.1	0.451	0.442
G:F	0.716	0.728	0.728	0.718	0.0059	0.852	0.085
d 0 to 42							
ADG, g	418	411	410	410	7.0	0.508	0.567
ADFI, g	562	552	546	551	9.2	0.448	0.316
G:F	0.742	0.745	0.751	0.744	0.0054	0.814	0.313

[1]A total of 360 pigs (PIC C-29 × 359) with 10 pigs per pen and 9 replications per treatment were used in a 42-d growth trial.

[2]*Lactobacillus plantarum* (LactoPlan; Nutraferma, Sioux, City, IA

Chapter 2 - Evaluating the effects of fish meal source and level on growth performance of nursery pigs

ABSTRACT

Three experiments were conducted to determine the effects of fish meal source on nursery pig growth performance. In Exp. 1, 250 pigs (PIC 327 × 1050, initially 7.1 kg and 5 d post-weaning) were fed either a corn-soybean meal-based diet, a diet containing 8.3% enzymatically treated soybean meal (HP 300 Hamlet Protein, Findlay, OH), or diets containing 6% fish meal from 1 of 3 sources (IPC 790, The Scoular Company, Minneapolis, MN; Special Select Menhaden, Omega Proteins, Houston, TX; LT Prime Menhaden, Daybrook Fisheries Inc., New Orleans, LA; source 1, 2, and 3, respectively). There were 5 pigs per pen and 10 pens per treatment with diets fed for 13-d. There was no evidence for differences among pigs fed any of the fish meal sources for ADG or ADFI; however, pigs fed fish meal source 1 had a marginally significant decreased (P =0.068) G:F compared with pigs fed diets with other protein sources. In Exp. 2, 350 barrows (DNA Line 200 × 400; initially 6.5 kg and 7 d post-weaning) were assigned to 1 of 7 dietary treatments and included the same control diet and 3 sources of fish meal used in Exp. 1, but fed at 3 or 6%. There were 5 pigs per pen and 10 pens per treatment with diets fed for 14-d. A source × level interaction (linear, $P < 0.05$) for ADG and G:F was observed as increasing fish meal source 1 increased ADG and G:F; however, pigs fed fish meal source 2 had improved ADG and G:F at 3%, but decreased performance at 6%. Pigs fed fish meal source 3 had no further improvements in ADG or G:F beyond the 3% inclusion. Fishmeal analysis for total volatile N, and modified torry digestibility did not appear to correspond with any growth performance differences measured in Exp. 1 or 2. In Exp. 3, 700 barrows (DNA Line 200 × 400, initially 6.2 kg and 3-d post-weaning) were fed a control diet or 4 diets with 6% fish meal

(source 3) containing either 0.87, 8.70, 16.52, or 24.35% fish solubles. There were 5 pigs per pen and 28 pens per treatment. Overall, pigs fed diets with fish meal had increased ($P < 0.05$) ADG and ADFI compared to pigs fed the control diet. There was no evidence for differences in growth performance as fish solubles increased. In conclusion, these data suggest inconsistencies in growth responses were observed with different fish meal sources, but the amount of fish solubles, total volatile N, or modified torry digestibility does not appear to explain these differences.

Key words: fish meal, fish solubles, growth, nursery pig

INTRODUCTION

To encourage feed intake for newly weaned pigs, highly palatable and nutrient dense protein sources, such as fish meal, are commonly added to nursery diets. Fish meal is typically considered a very good protein source due to its balance of AA, vitamins and minerals, and presence of omega 3 fatty acids (Church and Kellems, 1998; Li et al., 2014). However, the quality of fish meal used can vary considerably based on the species of fish, freshness of the raw material, and processing method (Pike, 1990). Because of these factors, growth responses to fish meal have sometimes been inconsistent (Kim and Easter, 2001; Jones et al., 2010).

One explanation of the inconsistencies of fish meal may reflect the amount of fish solubles added back into the presscake during the manufacturing process of whole fish meal. Fish solubles are a by-product derived from the intermediate fraction generated during the manufacturing process of fish meal and oil (Soares et al., 1972). Traditionally, fish solubles have been used directly as protein source or palatability enhancer in aquaculture diets (Hertrampf and Piedad-Pascual, 2000). Fish meal produced and sold today on average contains 8 to 15% fish solubles (Herbert, 2016). It is unclear if the amount of fish solubles contained within fish meal

will influence growth performance of pigs. Therefore, the objectives of these studies were to evaluate the growth performance of nursery pigs fed different sources of fish meal and determine if differences in growth performance are related to fish solubles added back to the fish meal during processing.

MATERIALS AND METHODS

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments. These experiments were conducted at the K-State Swine Teaching and Research Center (Exp. 1) and K-State Segregated Early Weaning facilities (Exp. 2 and 3). Each pen (1.52 × 1.22 m, Exp. 1; 1.22 × 1.22 m, Exp. 2 and 3) contained a 4-hole dry self-feeder and either a nipple waterer (Exp. 1) or cup waterer (Exp. 2 and 3) for ad libitum access to feed and water. All diets were fed in meal form and prepared at the O. H. Kruse Feed Technology and Innovation Center located in Manhattan, KS.

Experiment 1

A total of 250 pigs (327 × 1050 PIC, Hendersonville, TN; initially 7.1 kg) were used in a 13-d growth trial with 5 pigs per pen and 10 pens per treatment. Pigs were weaned at approximately 21 d of age, placed in nursery pens according to BW and fed a common pelleted starter diet for 5 d, at which time pigs were weighed and pens were allotted to 1 of 5 dietary treatments in a completely randomized design. Dietary treatments included a corn-soybean meal-based control diet, a diet containing 8.3% enzymatically treated soybean meal (ESBM; HP 300, Hamlet Protein, Findlay, OH), or diets with 6% fish meal from 1 of 3 sources (IPC 790, The Scoular Company, Minneapolis, MN; Special Select Menhaden, Omega Proteins, Houston, TX; LT Prime Menhaden, Daybrook Fisheries Inc., New Orleans, LA; sources 1, 2, and 3,

respectively; Table 2-1). Fish meal source 2 was from the 2014 catch year, while sources 1 and 3 were from the 2015 catch year. Diets (Table 2-2) were formulated such that 6% fish meal provided the same amount of standardized ileal digestible (SID) Lys as 8.3% ESBM. Calculated AA values (NRC 2012) and SID coefficients were used in diet formulation for the 3 fish meal sources, while nutrient values for the ESBM were provided by the manufacturer. Pigs and feeders were weighed on d 0, 7, and 13 of the trial to determine ADG, ADFI, and G:F.

Experiment 2

A total of 350 barrows (Line 200 × 400 DNA, Columbus, NE; initially 6.5 kg) were used in a 14-d growth trial with 5 pigs per pen and 10 pens per treatment. Pigs were weaned at approximately 21 d of age, placed in nursery pens according to BW and fed a common pelleted starter diet for 7 d, at which time pigs were weighed and pens were allotted to 1 of 7 dietary treatments in a complete randomized design. Dietary treatments (Table 2-4) included the same control diets and diets with fish meal from the same three sources, but different batches as those used in Exp. 1. Additionally, diets with 3% fish meal from the same sources were also included in this trial. Pigs and feeders were weighed on d 0, 7, and 14 of the trial to determine ADG, ADFI, and G:F.

Experiment 3

Two groups of 350 barrows (700 total; Line 200 × 400 DNA, Columbus, NE; initially 6.5 kg) were used in a 21-d growth trial with 5 pigs per pen and 14 pens per treatment in each group (28 total pens per treatment). Pigs were weaned at approximately 21 d of age, placed in nursery pens according to BW and fed a common pelleted starter diet for 3 d, at which time pigs were weighed and pens were allotted to 1 of 5 dietary treatments in a complete randomized block design. Dietary treatments included a control that was corn-soybean meal-based and 4 diets

containing 6% fish meal (source 3) with 0.87, 8.70, 16.52, and 24.35% fish solubles included in the fish meal.

Two batches of fish meal were used for this experiment to form the fish meal treatments. One fish meal batch contained 0.87% solubles and the second batch contained 24.35% solubles. A composite sample from each batch of fish meal was collected and analyzed for AA content and proximate analysis prior to formulation to determine nutrient loading values (Table 2-5). Then, basal diets containing the 0.87% and 24.35% solubles fish meal were manufactured and then blended to create the intermediate diets (Table 2-7). Diets were formulated to contain 1.35% SID Lys and balanced on an NE basis by lowering the choice white grease when fish meal was added. Net energy values from the 2012 NRC were used for the high solubles as the fat level closely resembled the analyzed fat level in the high solubles than the low solubles fish meal. For the low solubles fish meal, we calculated the difference in the amount of fat coming from the fish meal and added that amount of extra fat to the low solubles diet to equalize added fat from choice white grease and fish meal. Pigs and feeders were weighed on d 0, 7, 14, and 21 of the trial to determine ADG, ADFI, and G:F.

Diet Sampling and Analysis

Complete diet samples were obtained from feeders, composited, and frozen at -20ºC for subsequent analysis. Samples of ESBM and fish meal sources were collected at the feed mill at the time of feed manufacturing. Composite samples of diets, ESBM, and fish meal were split using a riffle splitter (Humboldt Mfg. Co., Norridge, IL) and processed through a 1 mm screen in a Willey mill (Thomas Scientific, Swedesboro, NJ) prior to analysis. All samples of diets and protein sources were submitted (Ward Laboratories Inc., Kearney, NE) for analysis of DM (method 935.29; AOAC International, 2012), CP (method 990.03; AOAC International., 2012),

ether extract (method 920.39; AOAC International, 2012) for preparation and analyzed using an ANKOM XT20 Fat Analyzer (Ankom Technology, Fairport, NY), Ca and P (method 968.08; AOAC International, 2012) for preparation using ICAP 6500 (ThermoElectron Corp., Waltham, MA), and ash (method 942.05; AOAC International, 2012). Samples of ESBM and fish meal used in all experiments were analyzed for their complete AA profile (method 982.30; AOAC International, 2006) by the University of Missouri-Columbia College of Agriculture Experiment Station Chemical Laboratories (Columbia, MO). Fish meal samples were submitted to New Jersey Feed Laboratories, Inc., (Trenton, NJ) for analysis of modified Torry digestibility (method 971.09 - 0.0002% pepsin; AOAC International, 2005), total volatile N analysis (method 971.09; AOAC International, 2005). Biogenic amines (method by CSL Food Science Lab, Torry, Aberdeen Scotland) were also measured for source 3 from Exp. 3 by New Jersey Feed Laboratories, Inc., (Trenton, NJ).

Statistical Analysis

Data were analyzed using the PROC GLIMMIX procedure in SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit and dietary treatment as a fixed effect. Data were analyzed as a complete randomized design for Exp. 1 and 2. For Exp. 1, treatment means were analyzed using the LSMEANS statement of SAS (SAS Institute, Inc., Cary, NC), with least squares means calculated for each independent variable. In Exp. 2, the main effects of source and level, as well as their interactions were tested. In Exp. 3, data were analyzed as a randomized complete block design with block (group) serving as the random effect in the model. Preplanned linear and quadratic contrasts were used to determine effects of increasing fish solubles on performance criteria. Results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

RESULTS

Chemical Analysis

Fish meal sources used in Exp. 1, 2, and 3 were high quality as indicated by the low total volatile N concentration (Tables 2-1, 2-3, and 2-5). Total volatile N was similar among fish meal sources. Fish meal source 2 used in Exp. 1 and 2 contained less CP and Lys than other sources with the largest difference from the calculated values used in formulation being observed in Exp. 1 compared with Exp. 2. Despite these differences, chemical composition of the complete diets was within analytical variation of their estimated values (Table 2-8 and 2-9).

Pepsin digestibility were similar (Table 2-5) between the low soluble and high soluble fish meal used in Exp. 3 with the high soluble fish meal having a higher modified Torry digestibility than the low soluble fish meal (92.4 vs. 86.4%). The low soluble fish meal had a higher CP content and concentrations of AA, but lower ether extract than the high soluble fish meal. Biogenic amine concentrations (Table 2-6) were lower in the low soluble fish meal compared to the high soluble fish meal.

Experiment 1

There was no evidence for differences among pigs fed any of the dietary treatments for ADG or ADFI (Table 2-11). However, pigs fed fish meal source 1 had a marginally significant reduction in overall G:F (P =0.068) compared to pigs fed diets with other protein sources.

Experiment 2

Overall, a source × level interaction (linear, $P < 0.05$) for ADG, G:F, and final BW was observed as increasing fish meal source 1 improved ADG and G:F; however, pigs fed fish meal source 2 had improved ADG and G:F at 3%, but decreased performance at 6% inclusion. Pigs

fed fish meal source 3 had no further improvements in ADG and G:F beyond the 3% inclusion. No evidence for differences was detected between the dietary treatments for ADFI.

Experiment 3

Overall, pigs fed diets with fishmeal had increased ($P < 0.05$) ADG, ADFI, and final BW compared to pigs fed the control diet without fish meal. There was no evidence for differences detected for growth performance when the amount of fish solubles was increased.

DISCUSSION

To encourage feed intake post-weaning, highly palatable and nutrient dense protein sources are often included in nursery diets. Historically, research has observed that including fish meal in early nursery diets improves growth performance and health (Stoner et al., 1990; Bergstrom et al., 1997). However, the magnitude of the growth response observed when feeding fish meal in nursery diets can be inconsistent (Kim and Easter, 2001; Jones et al., 2010).

Stoner et al. (1990) reported that the addition of 4 to 8% select Menhaden fish meal improved growth performance when replacing SBM. Similarly, Young et al. (2002) conducted an experiment in which pigs (~6.4 kg) were fed two sources of fish meal included at either 2.5 or 5%. The authors reported a linear improvement in ADG when pigs were fed increasing levels of fish meal. In contrast, Jones et al. (2010) reported that 3% select Menhaden fish meal was optimal to marginally improve ADG and ADFI; however, when pigs were fed either 5 or 6% fish meal, performance was similar to pigs fed a standard corn-soybean meal control diet with no specialty protein sources added. Our results from Exp. 1 and Exp. 2 also found inconsistencies in growth responses among fish meal sources.

The reasons for the lack of response when feeding 6% fish meal in Exp. 1 and pigs only fed source 1 having further improvements in ADG and G:F when 6% was included in Exp. 2 are

unclear. Traditional measurements for determining the freshness and quality of fish meal (total volatile N and modified Torry digestibility) were measured in both studies. These tests are designed as indicators of the degrees of freshness of the raw fish used in the manufacturing process and protein quality of the finished product, respectively. The total volatile N analysis measures free N, which is an indication of volatilization of crude protein (Kjeldsen et al., 1983). A value less than 0.15% is thought to indicate that the fish meal is of good quality (Kjeldsen et al., 1983). Whereas, the modified Torry digestibility is calculated as a portion of acid insoluble N that is soluble in acid pepsin solution (Bimbo, 1998). Biogenic amine concentrations were lower in the low soluble fish meal compared to the high soluble fish meal; however, these values did not change significantly during the extended storage period (5 months) between the groups of pigs that were used for the feeding trials. Thus, this would suggest that the product was stable as biogenic amines are produced as result of the degradation of AA via bacterial AA decarboxylases overtime (Opstvedt, et al., 1996). Based on these findings, chemical analyses did not explain the differences in performance found with the fish meal sources as total volatile N and modified Torry digestibility values were similar among fish meal sources and indicated fish meal of high quality. Noticeable differences in the nutrient composition between the sources of fish meal and formulated values used in Exp. 1 and 2 were observed. The reason for the differences between analyzed and formulated values are most likely due to the fact that formulated values based on the 2012 NRC are the average nutrient composition across various species of fish. This is important to note, due to previous research findings indicating that the nutrient composition varies greatly from species to species and varies based on age of the fish, environments in which the fish are reared, and season among others (Huss, 1995; Olsson et al., 2003; Boran et al., 2011).

In our study, Peruvian Anchovy (*Engraulis ringens*) fish were used in the manufacturing of source 1 fish meal; whereas, source 2 and 3 were derived from Gulf Menhaden (*Brevoortia patronus*). In addition, source 3 was dried at 70° C as opposed to the traditional 90° C. The reduction in drying temperature has been demonstrated to reduce the risk of negatively influencing protein quality (Pike et al., 1990; Ariyawansa, 2000). This was particularly relevant in an experiment conducted by Kim and Easter (2001) where the nutritional values of four fish meal sources (Menhaden, Mackerel – dried at 85°C, Mackerel – dried at 70°C, and Herring – dried at 70°C) were fed to nursery pigs for 4 weeks. The authors reported that apparent ileal AA digestibilities of all AA were 16 and 11% greater for Mackerel and Herring fish meal dried at 70°C, respectively, than Mackerel fish meal dried at 85°C. Whereas, apparent ileal AA digestibilities of all AA were on average 14 and 11% higher for Herring and Mackerel fish meal, respectively, than Menhaden fish meal. Consequently, species of fish used to produce the fish meal may influence the fish meal composition and may lead to different growth performance responses when fish meal is fed to weanling pigs.

Fish solubles (sometimes known as stickwater concentrate) are a by-product derived from the intermediate fraction (liquid phase) during the manufacturing process of fish meal (Wu and Bechtel, 2012). Fish solubles contain various water soluble and insoluble fractions that are rich sources of B vitamins and minerals (Soares et al., 1973). For this reason, the value of collecting and reincorporating solubles into the final product is of importance, but can also be expensive to recover due the viscous nature of the solubles. Therefore, the solubles must be treated either with a combination of acids or enzymes to hydrolyze the suspended and dissolved proteins, which enhances the evaporator and drying performance used in collecting the soluble fractions (Wu and Bechtel, 2012). Fish solubles are then added back into the fish meal to produce what is referred

to as whole fish meal. Fish meal commonly produced and sold today on average contains 8 to 15% fish solubles in the final product (Herbert, 2016).

Early work conducted by Laksesvela (1958) examining fish solubles and their relative feeding value to chicks indicated that solubles were a negligible protein source alone, but when fed in combination with presscake fish meal, feed intake was increased. Furthermore, Hulan and Proudfoot (1987) reported improved growth performance when broilers were fed a diet containing fish meal with added fish solubles compared to broilers that were fed fish meal with no added fish solubles. In addition, fish solubles have been used extensively as a protein source in aquaculture diets as an attractant/palatability enhancer to increase feed intake (Hertrampf and Piedad-Pascual, 2000; Kousoulaki et al., 2009).

Ours is the first study that we are aware of to determine the influence of fish solubles contained within fish meal on growth performance of pigs. In contrast to the poultry and aquaculture studies cited above, we observed no significant differences when fish solubles inclusion in fish meal ranged from 0.87 to 24.35% when 6% fish meal was included in the diet. It is unclear if swine are less sensitive to increasing fish solubles or if the fact that Hulan and Proudfoot (1987) did not account for the increasing AA contributed by the increased fish solubles allowed them to observe the improved performance with increased fish solubles. Nevertheless, our study would indicate that the response to fish meal is not dependent on the amount of fish solubles added to the fish meal.

In conclusion, based on the total volatile N analyses, modified Torry digestibility and total poly amine values, all fish meal sources tested were of high quality. Still, differences in growth performance were observed for pigs offered different amounts or sources of fish meal. It

is unclear why these differences in response were observed; however, it does not appear to be a reflection of the levels of fish solubles in the whole fish meal included in the diets.

LITERATURE CITED

AOAC International. 2005. Official Methods of Analysis of AOAC Int. 18th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

AOAC International. 2012. Official Methods of Analysis of AOAC Int. 19th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

Ariyawansa, S. 2000. The evaluation of functional properties of fish meal. Fisheries training program Reykjavik, Iceland. The United Nations University, Tokyo. p 1-24.

Bergstrom, J. R., J. L. Nelssen, M. D. Tokach, R. D. Goodband, S. S. Dritz, K. Q. Owen, and W. B. Nessmith, Jr. 1997. Evaluation of spray-dried animal plasma and select menhaden fish meal in transition diets of pigs weaned at 12 to 14 days of age and reared in different production systems. J. Anim. Sci. 75:3004-3009. doi:10.2527/1997.75113004x.

Bimbo, A. P. 1998. An evaluation of pepsin digestibility methods as practiced and interpreted by IFOMA and associated laboratories. In: Int. Fishmeal Oil Mfr. Assoc. London, UK.

Boran, G., and H. Karaçam. 2011. Seasonal changes in proximate composition of some fish species from the Black Sea. Turk. J. Fish Aquat. Sci. 11:1-5. doi: 10.4194/trjfas.2011.0101.

Church, D. C., and R. O. Kellems. 1998. Supplemental protein sources. In: R. O. Kellems and D. C. Church, editors, Livestock Feed and Feeding. Prentice Hall, Upper Saddle River, NJ.

Herbert, S. 2016. Personal communication.

Hertrampf, J. W., and F. Piedad-Pascual. 2000. Fish Solubles (De-hydrated). In: J. W. Hertrampf and F. Piedad-Pascual, editors, Handbook on Ingredients for Aquaculture Feeds. Springer Int. Pub., Cham, Switzerland.

Hulan, H. W., and F. G. Proudfoot. 1987. The effect of adding white fish meal containing enzyme digested or untreated stickwater solids to diets for broiler chickens. Anim. Feed. Sci. Tech. 16:253-259. doi: 10.1016/0377-8401(87)90014-9.

Huss, H. H. 1995. Quality and quality changes in fresh fish. FAO. Rome. P. 348.

Jones, C. K., J. M. DeRouchey, J. L. Nelssen, M. D. Tokach, S. S. Dritz, and R. D. Goodband. 2010. Effects of fermented soybean meal and specialty animal protein sources on nursery pig performance. J. Anim. Sci. 88:1725-1732. doi: 10.2527/jas.2009-2110.

Kjeldsen, N. J., V. Daniel. A. Just, H. E. Nielsen, and B. O. Eggum. 1983. Inclusion of fish meal manufactured from fish with different degrees of freshness in diets for early weaned pigs. Natl. Inst. Anim. Sci., Copenhagen Newsletter No. 390.

Kim, S. W., and R. A. Easter. 2001. Nutritional value of fish meals in the diet for young pigs. J. Anim. Sci. 79:1829-1839. doi:/2001.7971829x.

Kousoulaki, K., S. Albrektsen, E. Langmyhr, H. J. Olsen, P. Campbell, and A. Ksnes. 2009. The water soluble fraction in fish meal (stickwater) stimulates growth in Atlantic salmon (*Salmo salar* L.) given high plant protein diets. Aqua. 289:74-83 doi: 10.1016/j.aquaculture.2008.12.034.

Laksesvela, B. 1958. Protein value and amino-acid balance of condensed herring solubles and spontaneously heated herring meal. Chick experiments. J. Agric. Sci. 51:164-176. doi: 10.1017/S002185960003416.

Li, Q., J. H. Brendemuhl, K. C. Jeong, and L. Badinga. 2014. Effects of omega-3 polyunsaturated fatty acids on growth and immune response of weanling pigs. J. Anim. Sci. Technol. 56:7. doi: 10.1186/2055-0391-56-7.

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Olsson, G. P., R. L. Olsen, M. Carlehög, R. Ofstad. 2003. Seasonal variations in chemical and sensory characteristics of farmed and wild Atlantic halibut (Hippoglossus hippoglossus). Aqua. 217:191-205. doi: 10.1016/S0044-8486(02)00191-6.

Opstvedt, J., H. Mundheim, and H. Nygaard. 1996. Freshness of fish used in making fish meal for salmonids and the effects of biogenic amines. In: Int. Fishmeal & Oil Mfr. Assoc. London, UK.

Pike, I. H., G. Andorsdóttir, and H. Mundheim. 1990. The role of fish meal in diets for salmonids. In: 1990 Inter. Assoc. Fish Meal Manuf. No. 24. p. 1-41.

Soares, J., D. Miller, S. Cuppett, and P. Baursfeld. 1973. A review of the chemical and nutritive properties of condensed fish solubles. In: 1973 Fishery Bulletin Vol. 71 No. 1. p. 255-265.

Stoner, G. R., G. L. Allee, J. L. Nelssen, M. E. Johnston, and R. D. Goodband. 1990. Effect of select menhaden fishmeal in starter diets for pigs. J. Anim. Sci. 68:2729-2735. doi:/1990.6892729x.

Wu, T. H. and P. J. Bechtel. 2012. Screening for low molecular weight compounds in fish meal solubles by hydrophilic interaction liquid chromatography coupled to mass spectrometry. Food Chem. 130:739-745. doi:10.1016/j.foodchem.2011.05.088.

Young, M. G., M. D. Tokach, R. D. Goodband, J. L. Nelssen S. S. Dritz, and M. Cici. 2002. Comparison of International Corporation 740 fish meal and Special Select menhaden fish meal in nursery pig diets. J. Anim. Sci. 80(Suppl.2):71 (Abstr.).

Table 2-1. Chemical analysis of protein sources, Exp. 1 (as-fed basis)[1]

		Fish meal source		
Item	ESBM[2]	1[3]	2[4]	3[5]
Proximate analysis, %				
DM[6]	92.08	90.68	91.72	91.66
CP[6]	55.8	66.5	61.9	64.1
Ca[6]	0.27	3.88	5.85	5.38
P[6]	0.72	2.45	3.07	3.04
Ether extract[6]	1.0	7.3	9.1	7.6
Ash[6]	6.14	15.90	19.77	19.02
Total volatile N[7]	-	0.11	0.15	0.08
Modified Torry digestibility[7]	-	86.7	70.6	83.4
Total AA, %[8]				
Arg	3.85	3.63	3.67	3.79
Cys	0.72	0.57	0.41	0.55
His	1.31	1.95	1.09	1.37
Ile	1.89	2.20	1.75	2.07
Leu	3.91	4.66	3.60	4.42
Lys	3.25	5.02	3.86	4.82
Met	0.72	1.84	1.46	1.84
Phe	2.57	2.56	2.09	2.40
Thr	2.07	2.74	2.30	2.67
Trp	0.82	0.80	0.54	0.75
Tyr	2.01	2.04	1.61	1.95
Val	2.03	2.69	2.23	2.53

[1]Samples of protein sources were obtained at the mill during diet manufacturing.

[2]Hamlet Protein, Findlay, OH.

[3]IPC 790 (The Scoular Company, Minneapolis, MN).

[4]Omega Special Select Menhaden (Omega Protein, Houston, TX).

[5]LT Prime Menhaden (Daybrook Fisheries, Inc., New Orleans, LA).

[6]Ward Laboratories, Inc., (Kearney, NE)..

[7]New Jersey Feed Laboratory, Trenton, NJ.

[8]Amino acid analysis for protein sources was analyzed by the University of Missouri-Columbia College of Agriculture, Food and Natural Resources – Agriculture Experiment Station Chemical Laboratories, Columbia, MO.

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

Ingredient, %	Control	ESBM	Fish meal[2]
Corn	40.55	41.53	44.86
Soybean meal, 46.5%	32.75	23.36	23.37
Corn DDGS[3]	10.00	10.00	10.00
Spray-dried whey	10.00	10.00	10.00
Fish meal	---	---	6.00
ESBM[4]	---	8.30	---
Choice white grease	3.00	3.00	3.00
Limestone	1.05	1.10	0.78
Monocalcium P, 21% P	1.05	1.15	0.35
Sodium chloride	0.30	0.30	0.30
L-Lys HCl	0.35	0.35	0.35
DL-Met	0.15	0.15	0.14
L-Thr	0.11	0.10	0.13
L-Trp	---	---	0.03
L-Val	0.03	---	0.05
Phytase[5]	0.02	0.02	0.02
Zinc oxide	0.25	0.25	0.25
Trace mineral premix[6]	0.15	0.15	0.15
Vitamin premix[7]	0.25	0.25	0.25
TOTAL	100	100	100
Calculated analysis			
Standard ileal digestible (SID) amino acid, %			
Lys	1.35	1.35	1.35
Ile:Lys	64	62	61
Met:Lys	35	35	37
Met & Cys:Lys	58	58	58
Thr:Lys	63	63	63
Trp:Lys	18.5	18.5	18.5
Val:Lys	71	71	71
Total Lys, %	1.52	1.51	1.53
ME, kcal/kg	3,408	3,439	3,461
NE kcal/kg	2,509	2,535	2,571
SID Lys:ME, g/Mcal	3.96	3.92	3.90
CP, %	23.4	23.6	23.1
Ca, %	0.77	0.77	0.77
P, %	0.69	0.65	0.66
Available P, %	0.51	0.51	0.51

[1]Diets were fed from 7.1 to approximately 10.4 kg.

[2]Fish meal sources were: IPC 790 (2015 catch year, The Scoular Company, Minneapolis, MN); Omega Special Select Menhaden (2014 catch year, Omega Protein, Houston, TX); Daybrook LT Prime Menhaden (2015 catch year, Daybrook Fisheries, Inc., New Orleans, LA).

[3]Dried distillers grain with solubles.

[4] Enzymatically treatetd soybean meal; HP 300, amlet Protein, Findlay, OH.

[5]Ronozyme® HiPhos 2700 (DSM Nutritional Products, Parsippany, NJ) provided 476.2 phytase units (FTU/kg) of diet with a release of 0.10% available P.

[6]Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulphate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[7]Provided per kilogram of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 46 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

Table 2-3. Chemical analysis of fish meal sources, Exp. 2 (as-fed basis)[1]

Item	Formulated Values[2]	Fish meal source 1[3]	2[4]	3[5]
Proximate analysis, %				
DM[5]	93.70	91.07	89.64	91.72
CP[5]	63.28	66.53	57.83	62.46
Ca[5]	4.28	4.13	3.97	5.93
P[5]	2.93	2.48	2.51	2.78
Ether extract[6]	9.71	8.78	7.64	8.64
Ash[6]	16.07	17.43	16.45	18.46
Total volatile N[7]	---	0.13	0.10	0.09
Modified Torry digestibility[7]	---	91.70	85.20	89.10
Total AA, %[8]				
Arg	3.84	3.66	3.59	3.89
Cys	0.61	0.59	0.49	0.51
His	1.44	2.26	1.35	1.39
Ile	2.56	2.13	1.93	2.18
Leu	4.47	4.75	4.14	4.46
Lys	4.56	5.18	4.54	4.86
Met	1.73	1.86	1.66	1.80
Phe	2.47	2.57	2.29	2.38
Thr	2.58	2.79	2.54	2.64
Trp	0.63	0.87	0.65	0.63
Tyr	1.88	2.09	1.87	2.00
Val	3.06	2.62	2.37	2.67

[1]Samples of fish meal were obtained at the mill during diet manufacturing and composited. All fish meal sources were from the 2014 catch year.

[2]Formulated values: refers to values reported in the 2012 NRC.

[3]IPC 790 (The Scoular Company, Minneapolis, MN).

[4]Omega Special Select (Omega Protein, Houston, TX).

[5]LT Prime Menhaden (Daybrook Fisheries, Inc., New Orleans, LA).

[6]Ward Laboratories, Inc., Kearney, NE.

[7]New Jersey Feed Laboratory, Trenton, NJ.

[8]Amino acids were analyzed by the University of Missouri-Columbia College of Agriculture, Food and Natural Resources – Agriculture Experiment Station Chemical Laboratories, Columbia, MO.

Table 2-4. Diet composition, Exp. 2 (as-fed basis)[1]

		Fish meal[2]	
Ingredient, %	Control	3%	6%
Corn	40.55	42.70	44.86
Soybean meal, 46.5%	32.75	28.06	23.37
Corn DDGS[3]	10.00	10.00	10.00
Spray-dried whey	10.00	10.00	10.00
Fish meal	---	3.00	6.00
Choice white grease	3.00	3.00	3.00
Limestone	1.05	0.91	0.78
Monocalcium P, 21% P	1.05	0.70	0.35
Sodium chloride	0.30	0.30	0.30
L-Lys HCl	0.35	0.35	0.35
DL-Met	0.15	0.14	0.14
L-Thr	0.11	0.12	0.13
L-Trp	---	0.01	0.03
L-Val	0.03	0.04	0.05
Phytase[4]	0.02	0.02	0.02
Zinc oxide	0.25	0.25	0.25
Trace mineral premix[5]	0.15	0.15	0.15
Vitamin premix[6]	0.25	0.25	0.25
TOTAL	100	100	100
Calculated analysis			
Standard ileal digestible (SID) amino acid, %			
Lys	1.35	1.35	1.35
Ile:Lys	64	62	61
Met:Lys	35	36	37
Met & Cys:Lys	58	58	58
Thr:Lys	63	63	63
Trp:Lys	18.5	18.5	18.5
Val:Lys	71	71	71
Total Lys, %	1.52	1.53	1.53
ME, kcal/kg	3,408	3,435	3,461
NE NRC, kcal/kg	2,509	2,540	2,571
SID Lys:ME, g/Mcal	3.96	3.93	3.90
CP, %	23.4	23.2	23.1
Ca, %	0.77	0.77	0.77
P, %	0.69	0.68	0.66
Available P, %	0.51	0.51	0.51

[1]Diets were fed from 6.5 to approximately 10.2 kg.

[2]Fish meal sources were: IPC 790 (The Scoular Company, Minneapolis, MN); Omega Special Select fish meal (Omega Protein, Houston, TX); Daybrook LT Prime Menhaden Fishmeal (Daybrook Fisheries, Inc., New Orleans, LA). All fish meal sources were from the 2014 catch year.

[3]Dried distillers grain with solubles.

[4]Ronozyme® HiPhos 2700 (DSM Nutritional Products, Parsippany, NJ) provided 476.2 phytase units (FTU/kg) of diet with a release of 0.10% available P.

[5]Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulphate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[6]Provided per kilogram of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 46 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

Table 2-5. Chemical analysis of fish meal sources, Exp. 3 (as-fed basis)[1,2]

Item	0.87% soluble fish meal	24.35% soluble fish meal
Proximate analysis, %		
DM[3]	92.60	93.01
CP[3]	66.05	63.25
Ca[3]	7.07	5.17
P[3]	3.30	2.61
Ether extract[3]	6.95	10.61
Ash[3]	19.23	19.11
Total volatile N[4]	0.07	0.06
Pepsin digestibility[4]	94.37	93.29
Modified Torry digestibility[4]	86.4	92.4
Total AA, %[5]		
Arg	4.16	3.69
Cys	0.60	0.48
His	1.62	1.51
Ile	2.96	2.52
Leu	4.96	4.28
Lys	5.53	4.82
Met	1.95	1.68
Thr	2.78	2.40
Trp	0.76	0.61
Tyr	2.29	1.79
Val	3.50	3.09

[1]Samples of fish meal were obtained at the mill during diet manufacturing and composited.

[2]LT Prime Menhaden Fishmeal (Daybrook Fisheries Inc., New Orleans, LA).

[3]Ward Laboratories, Inc., Kearney, NE.

[4]New Jersey Feed Laboratory, Trenton, NJ.

[5]Amino acids were analyzed by the University of Missouri-Columbia College of Agriculture, Food and Natural Resources – Agriculture Experiment Station Chemical Laboratories, Columbia, MO.

Table 2-6. Biogenic amines concentrations of fish meal sources, Exp. 3 (as-fed basis)[1,2,3]

Item, ppm	0.87% soluble fish meal	24.35% soluble fish meal
Group 1		
Tyramine	6	130
Putrescine	11	135
Cadaverine	38	508
Histamine	4	134
Agmatine	28	181
Spermidine	24	42
Spermine	4	21
Group 2		
Tyramine	16	129
Putrescine	16	133
Cadaverine	52	483
Histamine	2	103
Agmatine	33	170
Spermidine	36	48
Spermine	21	14

[1] Two groups of 350 barrows (700 total; and 28 total pens per treatment) were used in a 21-growth trial. Group 2 was placed on test 5 months after group 1, thus, biogenic amines were tested on the same batch of fish meal to monitor the stability of the product over a 5 month storage period in 25 kg bags located in an unregulated environment subject to fluctuations in temperature and humidit.

[2]Samples of fish meal were obtained at the mill during diet manufacturing, composited, and submitted to New Jersey Feed Laboratory (Trenton, NJ) for analysis.

[3]LT Prime Menhaden Fishmeal (Daybrook Fisheries Inc., New Orleans, LA).

Table 2-7. Diet composition, Exp. 3 (as-fed basis)[1]

Ingredient, %	Control	Soluble fractions, %[2]	
		0.87	24.35
Corn	40.31	48.65	48.33
Soybean meal, 46.5% CP	32.77	21.35	21.35
Corn DDGS[3]	10.00	10.00	10.00
Spray-dried whey	10.00	10.00	10.00
Fish meal[4]	---	6.00	6.00
Choice white grease	3.00	1.45	1.25
Limestone	1.07	0.42	0.62
Monocalcium P, 21% P	1.05	0.25	0.45
Sodium chloride	0.50	0.50	0.50
L-Lys HCl	0.35	0.35	0.39
DL-Met	0.15	0.14	0.16
L-Thr	0.11	0.14	0.17
L-Trp	---	0.03	0.04
L-Val	0.03	0.06	0.08
Phytase[5]	0.02	0.02	0.02
Zinc oxide	0.25	0.25	0.25
Trace mineral premix[6]	0.15	0.15	0.15
Vitamin premix[7]	0.25	0.25	0.25
TOTAL	100	100	100
Calculated analysis			
Standardized ileal digestible (SID) amino acids, %			
Lys	1.35	1.35	1.35
Ile:Lys	64	60	58
Leu:Lys	131	127	124
Met:Lys	35	37	38
Met & Cys:Lys	58	58	58
Thr:Lys	63	63	63
Trp:Lys	18.5	18.5	18.5
Val:Lys	71	71	71
ME, kcal/kg	3,402	3,371	3,377
NE, kcal/kg	2,502	2,502	2,502
CP, %	23.4	22.7	22.6
Ca, %	0.78	0.78	0.78
P, %	0.69	0.66	0.66
Available P, %	0.51	0.51	0.51

[1]Diets were fed from 6.5 to approximately 13.1 kg .

[2]Treatments 0.87% and 24.35% solubles were manufactured and blended to create the intermediate levels of 8.70% and 16.52% solubles.

[3]Dried distillers grain with solubles.

[4]LT Prime Menhaden Fishmeal (Daybrook Fisheries Inc., New Orleans, LA).

[5]Ronozyme® HiPhos 2700 (DSM Nutritional Products, Parsippany, NJ) provided 476.2 phytase units (FTU/kg) of diet with a release of 0.10% available P.

[6]Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulphate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[7]Provided per kilogram of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 46 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

Table 2-8. Chemical analysis of diets, Exp. 1 (as-fed basis)[1]

			Fish meal source		
Item, %	Control	ESBM[2]	1[3]	2[4]	3[5]
DM	90.27	88.73	88.58	90.46	90.18
CP	24.20	24.20	22.30	24.00	23.20
Ca	0.81	0.89	0.84	0.89	0.89
P	0.71	0.73	0.64	0.69	0.72
Ether extract	5.70	5.10	5.50	5.40	5.60
Ash	6.11	5.36	5.76	5.73	6.21

[1]Complete diets were sampled at the feeder pooled, mixed, and then split using a riffle splitter to create a composite sample and submitted to Ward Laboratories (Kearney, NE) for analysis.

[2]HP 300 (Hamlet Protein, Findlay, OH).

[3]IPC 790 (The Scoular Company, Minneapolis, MN).

[4]Omega Special Select (Omega Protein, Houston, TX).

[5]LT Prime Menhaden (Daybrook Fisheries, New Orleans, LA).

Table 2-9. Chemical analysis of diets, Exp. 2 (as-fed basis)[1]

Item, %	Control	1[2]		2[3]		3[4]	
		3%	6%	3%	6%	3%	6%
DM	92.08	90.14	90.40	90.48	89.25	90.75	90.94
CP	24.80	24.70	24.20	24.50	23.90	23.30	23.70
Ca	0.81	0.76	0.87	0.81	0.92	0.78	0.87
P	0.73	0.77	0.70	0.71	0.66	0.69	0.68
Ether Extract	5.60	4.90	6.10	5.10	6.20	5.40	5.60
Ash	5.72	5.86	5.43	5.91	6.23	5.83	5.76

[1]Complete diets were sampled at the feeder pooled, mixed, and then split using a riffle splitter to create a composite sample and submitted to Ward Laboratories (Kearney, NE) for analysis.

[2]IPC 790 (The Scoular Company, Minneapolis, MN).

[3]Omega Special Select (Omega Protein, Houston, TX).

[4]LT Prime Menhaden (Daybrook Fisheries, New Orleans, LA).

Table 2-10. Chemical analysis of diets, Exp. 3 (as-fed basis)[1,2]

Item, %	Control[2]	Soluble fractions, %[3,4]			
		0.87	8.70	16.52	24.35
DM	89.04	88.94	89.30	89.64	89.56
CP	22.7	22.6	21.6	22.6	22.3
Ca	1.15	0.82	0.77	0.93	0.81
P	0.81	0.72	0.70	0.78	0.77
Ether extract	4.7	4.2	4.2	4.8	4.8
Ash	6.50	5.62	5.59	6.02	5.86

[1]Complete diets were sampled at the feeder pooled, mixed, and then split using a riffle splitter to create a composite sample and submitted to Ward Laboratories (Kearney, NE) for analysis.

[2]The control diet contained no fish meal.

[3]Treatments 0.87% and 24.35% solubles were manufactured and blended at O. H. Kruse Feed Technology and Innovation Center to create the intermediate levels of 8.70% and 16.52% solubles.

[4]LT Prime Menhaden Fishmeal (Daybrook Fisheries Inc., New Orleans, LA).

Table 2-11. Effects of fish meal source on nursery pig performance, Exp. 1[1]

			Fish meal source				
Item	Control	ESBM[2]	1[3]	2[4]	3[5]	SEM	Probability, $P <$
BW, kg							
d 0	7.06	7.07	7.06	7.06	7.06	0.057	1.000
d 13	10.45	10.27	10.30	10.46	10.55	0.181	0.791
d 0 to 13							
ADG, g	261	247	249	262	269	11.6	0.652
ADFI, g	370	342	388	361	367	16.4	0.406
G:F	0.720[x]	0.732[x]	0.657[y]	0.730[x]	0.743[x]	0.0221	0.068

[xy]Means within the same row with different superscripts differ ($P < 0.10$).

[1]A total of 250 pigs (327 × 1050 PIC, Hendersonville, TN; initially 7.1 kg) were used in a 13-d growth trial with 5 pigs per pen and 10 replications per treatment.

[2]HP 300 (Hamlet Protein, Findlay, OH).

[3]IPC 790, 2015 catch year (The Scoular Company, Minneapolis, MN).

[4]Omega Special Select Menhaden, 2014 catch year (Omega Protein, Houston, TX).

[5]LT Prime Menhaden, 2015 catch year (Daybrook Fisheries Inc., New Orleans, LA).

Table 2-12. Effects of fish meal souce and level on nursery pig performance, Exp. 2[1,2,3]

		Fish meal source							Probability, $P <$	
		1[3]		2[4]		3[5]			Source × Level	
Item	CTRL	3%	6%	3%	6%	3%	6%	SEM	Linear	Quadratic
BW, kg										
d 0	6.49	6.51	6.49	6.50	6.49	6.51	6.50	0.091	0.996	0.998
d 14	10.07	10.23	10.52	10.40	9.87	10.26	10.19	0.176	0.039	0.207
d 0 to 14										
ADG, g	255	266	288	277	238	268	264	10.5	0.006	0.110
ADFI, g	329	344	354	349	330	332	335	11.3	0.303	0.493
G:F	0.774	0.777	0.811	0.793	0.725	0.808	0.790	0.0201	0.010	0.171

[1]A total of 350 maternal line barrows (200 × 400 DNA, Columbus, NE; initially 6.5 kg) with 5 pigs per pen and 10 replications per treatment were used in a 14-d growth trial.

[2]No evidence for differences were detected for the main effects of source or level

[2]All fish meal sources were from the 2014 catch year.

[3]IPC 790 (The Scoular Company, Minneapolis, MN).

[4]Omega Special Select Menhaden (Omega Protein, Houston, TX).

[5]LT Prime Menhaden (Daybrook Fisheries, Inc., New Orleans, LA).

Table 2-13. Effects of increasing fish solubles on nursery pig performance, Exp. 3[1]

							Probability, $P <$		
		Soluble fractions, %[2]					Control vs.	Soluble fractions	
	Control[3]	0.87	8.70	16.52	24.35	SEM	Fishmeal	Linear	Quadratic
BW, kg									
d 0	6.49	6.49	6.50	6.50	6.49	0.274	0.568	0.914	0.180
d 21	12.70	13.24	13.06	13.36	13.33	0.147	<0.001	0.332	0.566
d 0 to 21									
ADG, g	293	322	309	322	321	14.9	0.001	0.704	0.395
ADFI, g	412	442	431	447	449	13.9	0.001	0.282	0.424
G:F	0.711	0.729	0.717	0.722	0.716	0.0133	0.258	0.341	0.740

[1]A total of 700 maternal line barrows (200 × 400 DNA, Columbus, NE; initially 6.5 kg) with 5 pigs per pen and 28 replications per treatment were used in 21-d growth trial.

[2]The control diet contained no fishmeal.

[3]Two batches of LT Prime Menhaden Fishmeal were manufactured with 0.87 and 24.35% soluble fractions (Daybrook Fisheries Inc., New Orleans, LA). Treatment diets with 0.87 and 24.35% solubles were then blended to create the intermediate diets with 8.70 and 16.52% solubles that were all added at 6% to the diet.

Chapter 3 - Evaluating the effects of enzymatically-treated soybean meal on nursery pig performance

ABSTRACT

Two experiments were conducted to evaluate the effects of enzymatically treated soybean meal on nursery pig growth performance. In Exp. 1, 1,215 weanling pigs (PIC 337 × 1050; initially 5.10 kg) were allotted to 1 of 5 dietary treatments in a 43-d study with 27 pigs per pen and 9 pens per treatment. Experimental diets were fed in two phases from d 0 to 7 and 7 to 22, followed by a common diet from d 22 to 43. The treatments included a control diet that was a standard corn soybean meal-based diet with 7.5% and 5.6% fish meal in phase 1 and 2, respectively. Diets 2 to 4 contained increasing enzymatically-treated soybean meal (ESBM; HP 300; Hamlet Protein, Findlay, OH) ranging from 6.7 to 20% in phase 1, and 5 to 15% in phase 2. The fifth treatment had the same amount of SBM as the control diet, but with ESBM replacing fish meal. From d 0 to 22, increasing ESBM decreased (linear, $P < 0.05$) ADG and ADFI; however, there were no differences observed for G:F. No evidence for differences were observed among pigs fed the fish meal control diet and pigs fed the ESBM diet replacing only fish meal. Overall (d 0 to 43), pigs fed increasing ESBM had a marginally significant decreased ADFI (linear, $P = 0.071$) and final BW (linear, $P = 0.043$). No differences were observed for growth performance among pigs fed the fish meal control diet and pigs fed ESBM replacing only fish meal. In Exp. 2, 350 barrows (DNA Line 200 × 400; initially 6.2 kg) were used for 21-d with 5 pigs per pen and 14 pens per treatment. The 5 corn-soybean meal based treatment diets were 1) soybean meal control (no specialty protein products); 2) diet with 6% fish meal; 3) diet with 9.1% ESBM replacing fish meal on a Lys basis; 4) diet with 6% ESBM replacing fish meal on a kg/kg basis, and 5) diet with 15% ESBM included at the expense of SBM and fish meal. Overall

(d 0 to 21), ADG and ADFI decreased ($P < 0.10$ and $P < 0.05$, respectively) when pigs were fed 15% ESBM compared with pigs fed the fish meal control diet. Pigs fed the soybean meal control diet had the poorest G:F ($P < 0.05$) among the dietary treatments. Furthermore, pigs fed the fish meal control diet had increased final BW ($P < 0.05$) compared to pigs fed the soybean meal control, ESBM replacing fish meal on a SID Lys basis, and 15% ESBM diet, respectively. In conclusion, these data suggest that nursery pigs fed diets with increasing levels of the ESBM tested in these experiments resulted in decreased ADFI and final BW.

Key words: enzymatically treated soybean, fish meal, growth, nursery pig

INTRODUCTION

Conventionally processed soybean meal (SBM) is the most commonly used protein source fed to swine in the United States (Cromwell, 2000). However, SBM contains anti-nutritional factors that when fed in high amounts produces what is known as SBM delayed-type transient hypersensitivity (Li et al., 1990). This form of transient hypersensitivity results in abnormalities specifically at the cellular level in the gastrointestinal tract that include decreased villous height and hypertrophy of intestinal crypts that can result in poorer growth performance (Li et al., 1990; 1991). For this reason, highly digestible AA sources such as milk- and animal-based ingredients are frequently added to diets for weanling pigs. However, concerns with cost and bio-security related to porcine epidemic diarrhea virus (PEDv) contaminated ingredients have led to increased interest in the use of plant-based alternative protein sources.

One protein source that has shown potential for use to reduce SBM is enzymatically-treated soybean meal (ESBM; HP 300, Hamlet Protein, Findlay, OH). This particular source of ESBM is produced from soybean meal that has been treated with a proprietary blend of enzymes resulting in the reduction of anti-nutritional factors that may be found in conventional soybean

meal (Cervantes-Pahm and Stein, 2010). This may enhance its nutritional value and allow it to be incorporated at higher amounts relative to SBM in starter diets.

Therefore, the objectives of our studies were to evaluate the growth performance of nursery pigs: 1) fed increasing ESBM in a commercial research facility and 2) fed diets with ESBM as a replacement for fish meal.

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 a commercial research nursery in southwestern Minnesota. Each pen (3.65 × 2.29 m) had completely slatted plastic floors and was equipped with a 6-hole, stainless-steel, dry self-feeder, and a pan waterer for ad libitum access to feed and water. Daily feed additions to each pen were made and recorded by a computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN). Experiment 2 was conducted at the K-State Segregated Early Weaning facilities (Manhattan, KS). Pens (1.22 × 1.22 m) had metal tri-bar flooring and were equipped with a 4-hole, stainless-steel, dry self-feeder, and a cup waterer for ad libitum access to feed and water.

Experiment 1

A total of 1,215 pigs (337 × 1050 PIC, Hendersonville, TN; initially 5.10 kg) were used in a 43-d growth trial with 27 pigs per pen and 9 pens per treatment. Pigs were weaned at approximately 16 to 19 d of age and placed in pens with each pen containing an even mix of barrows and gilts. Pens of pigs were weighed and allotted by BW to 1 of 5 dietary treatments in a randomized complete block design. Experimental diets were fed in two phases from d 0 to 7 (Table 3-1) and 7 to 22 (Table 3-2), followed by a common phase 3 diet fed from d 22 to 43

(Table 3-2). The treatments included a control diet that was a standard corn SBM-based diet with 7.5% and 5.6% fish meal in phase 1 and 2, respectively. Diets 2 to 4 were formulated to contain increasing ESBM (HP 300; Hamlet Protein, Findlay, OH) ranging from 6.7 to 20% in phase 1, and 5 to 15% in phase 2 with an equally spaced increase in ESBM and reduction in fish meal. The fifth treatment had the same amount of SBM as the fish meal-control diet, but with ESBM replacing fish meal on an equal SID Lys basis. Phase 1 diets were fed in pellet form and manufactured at Hubbard Feeds (Worthington, MN), while phases 2 and 3 were fed in meal form and manufactured at New Horizon Farms (Pipestone, MN). Nutrient loading values used in diet formulation were provided by the manufacture. Standard ileal digestible (SID) coefficients were based on the NRC (2012). Pigs and feeders were weighed on d 0, 7, 14, 22, 29, 36, and 43 of the trial to determine ADG, ADFI, and G:F.

Experiment 2

A total of 350 maternal line barrows (Line 200 × 400 DNA, Columbus, NE; initially 6.2 kg) were used in a 21-d growth trial with 5 pigs per pen and 14 pens per treatment. Pigs were weaned at approximately 21 d of age, placed in nursery pens according to BW and fed a common pelleted starter diet for 3 d, at which time pigs were weighed and pens were blocked by BW to 1 of 5 dietary treatments in a randomized complete block design. A composite sample of fish meal and ESBM (HP 300; Hamlet Protein, Findlay, OH) was collected and analyzed for AA content and proximate analysis prior to formulation to determine nutrient loading values (Table 3-3). Standard ileal digestible (SID) coefficients were based on the NRC (2012). Dietary treatments were standard corn soybean-meal based with 10% spray-dried whey and formulated to contain 1.35% SID Lys and balanced on an NE basis. The 5 treatment diets (Table 3-4) were corn SBM-based and consisted of: 1) a negative control (no specialty protein products); 2) a diet with 6%

fish meal; 3) a diet with 9.1% ESBM replacing fish meal on a SID Lys basis; 4) a diet with 6% ESBM replacing fish meal on a kg/kg basis, and 5) a diet with 15% ESBM included at the expense of SBM and fish meal. All diets were fed in meal form and prepared at the O. H. Kruse Feed Technology and Innovation Center located in Manhattan, KS. Pigs and feeders were weighed on d 0, 7, 14, and 21 of the trial to determine ADG, ADFI, and G:F.

Diet Sampling and Analysis

Complete diet samples were obtained from feeders, composited, and frozen at -20ºC for subsequent analysis. Samples of ESBM and fish meal were collected at the mill. Composite samples of diets, ESBM, and fish meal were split using a riffle splitter (Humboldt Mfg. Co., Norridge, IL) and processed through a 1 mm screen in a Willey mill (Thomas Scientific, Swedesboro, NJ) prior to analysis. All samples of diets and protein sources were submitted (Ward Laboratories Inc., Kearney, NE) for analysis of DM (method 935.29; AOAC International, 2012), CP (method 990.03; AOAC International., 2012), ether extract (method 920.39; AOAC International, 2012) for preparation and analyzed using an ANKOM XT20 Fat Analyzer (Ankom Technology, Fairport, NY), Ca and P (method 968.08; AOAC International, 2012) for preparation using ICAP 6500 (ThermoElectron Corp., Waltham, MA), and ash (method 942.05; AOAC International, 2012). Amino acid analysis for complete diet samples in Exp. 1 were analyzed by Ajinomoto Heartland Inc., (Chicago, IL; method 994.12; AOAC International, 2012). Samples of ESBM and fish meal used in Exp. 2 were analyzed for their complete AA profile (method 982.30; AOAC International, 2006) by the University of Missouri-Columbia College of Agriculture Experiment Station Chemical Laboratories (Columbia, MO).

Water holding capacity for complete diets as well as soybean meal, ESBM, and fish meal were determined using the centrifugation method described by Kyriazakis and Emmans (1995).

Each sample was assessed in duplicate with a coefficient of variation less than 10%. Flowability was measured using a Flowdex device (Hanson Research, Chatsworth, CA), which measures flowability based on an ingredients ability to fall freely through a hole in the center of a disk. In addition, flowability was measured using angle of repose in which the diet was placed in a cylinder on top of an 8.7 cm diameter pedestal. The cylinder was then lifted to allow the diet to fall freely. The height of the remaining diet was measured and used to calculate angle of repose.

Statistical Analysis

Data were analyzed using the PROC GLIMMIX procedure in SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit, dietary treatment as a fixed effect, and block serving as the random effect in the model. For Exp. 1, preplanned linear and quadratic polynomial contrasts were used to determine the effects of increasing ESBM on performance criteria. A pairwise comparison between the control and the diet with ESBM replacing fish meal was performed using the DIFFS option from the LSMEANS statement of SAS. In Exp. 2, treatment means were analyzed using the LSMEANS statement of SAS (SAS Institute, Inc., Cary, NC), with least squares means calculated for each independent variable. Results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

RESULTS

Chemical Analysis

In Exp. 1, results from the proximate analysis of experimental diets and protein sources showed that most nutrients were similar to formulated values for all three phases, with the exception of Ca in the phase 1 control diet which was higher than expected (Table 3-5). The AA analysis of complete diets for phase 1 were consistently lower than expected across dietary treatments; however, analyzed values for phase 2 generally matched formulated values. Analysis

of SBM, fish meal, and ESBM used in Exp. 1 indicated that ESBM had the greatest water holding capacity (Table 3-5). Water holding capacity increased as ESBM was included in diets at the expense of SBM and fish meal. Flowability was not able to be measured on phase 1 diets because they were pelleted. Flowability characteristics of diets fed in phase 2 were similar as indicated by similar flowability index scores using the Flowdex device as well as angle of repose. As expected, the trypsin inhibitor content of ESBM was lower than SBM. Proximate analysis for the phase 3 common diet closely matched formulated values (Table 3-6). In Exp. 2, dietary analysis indicated that most nutrients were similar to formulated values with the exception of Ca which were higher than formulated values across all dietary treatments.

Experiment 1.

From d 0 to 7, increasing ESBM at the expense of SBM and fish meal decreased then increased ADFI (quadratic, $P = 0.001$) as ESBM increased. No evidence for differences were observed for ADG or G:F. Furthermore, performance did not differ among pigs fed the fish meal control diet and pigs fed the diet with ESBM replacing fish meal. During d 7 to 22, ADG and ADFI decreased (linear, $P < 0.05$) as ESBM increased at the expense of SBM and fish meal resulting in a marginally significant poorer G:F (quadratic, $P = 0.061$). However, no differences were observed among pigs fed the fish meal control diet and pigs fed the diet where ESBM replaced fish meal.

From d 0 to 22, ADG, ADFI, and d 22 BW decreased (linear, $P < 0.05$) as ESBM increased. In addition, no evidence for differences were observed among pigs fed the fish meal control diet and pigs fed the ESBM diet replacing only fish meal. During the common period (d 22 to 43), a marginally significant improvement in G:F (quadratic, $P = 0.073$) was observed with pigs previously fed the diet with the low inclusion of ESBM having the best G:F. Overall (d 0 to

43), pigs fed increasing ESBM had a marginally significant reduction in ADFI (linear, P = 0.071) and decreased final BW (linear, P = 0.043). However, no differences were observed for growth performance among pigs fed the fish meal control diet and pigs fed the diet with ESBM replacing only fish meal.

Experiment 2.

Overall (d 0 to 21), ADG ($P < 0.10$), and ADFI decreased ($P < 0.05$) when pigs were fed 15% ESBM compared with pigs fed the fish meal control diet. Pigs fed the soybean meal control diet had the poorest G:F ($P < 0.05$) among the dietary treatments Furthermore, pigs fed the fish meal control diet had increased final BW ($P < 0.05$) compared to pigs fed the soybean meal control, ESBM replacing fish meal on a SID Lys basis, and 15% ESBM diet, respectively.

DISCUSSION

Conventionally processed SBM is considered an excellent protein source due to its balance of AA that complements the concentration of AA in cereal grains (NRC, 2012). In addition to its nutrient profile, its relative availability and low cost make it one of the most commonly used protein sources fed to swine in the United States (Cromwell, 2000). However, previous research has indicated that trypsin inhibitors present in soybeans reduce proteolytic enzyme activity resulting in a decrease in protein digestion (Yen et al., 1977). In addition, both glycinin and β-conglycinin have been shown to induce transient hypersensitivity resulting in abnormalities specifically at the cellular level in the gastrointestinal tract that include decreased villous height and hypertrophy of intestinal crypts that can lead to reduced growth performance (Li et al., 1990; 1991; Chen et al., 2011). Therefore, specialty-based protein sources are included to reduce the amount of soybean meal in starter diets (Bergstrom et al., 1997).

One specialty animal-based protein source that has been widely used in nursery diets is fish meal. In general, fish meal is a good source of amino acids, vitamins and minerals, and improved growth performance of weanling pigs (Stoner et al., 1990; Kim and Easter, 2001). However, the growth response to fish meal can be variable due to the quality of fish and processing factors used in the production of fish meal (Wiseman et al., 1991; Kim and Easter, 2001). Due to its variability and increasing cost, many producers have sought a more economical protein source to use. One protein source that has shown potential for use to replace SBM and/or fishmeal is ESBM. The ESBM used in these studies is a finely ground soy protein produced from SBM that has been treated with a proprietary blend of enzymes resulting in the reduction of anti-nutritional factors and an improvement in SID AA digestibility and P digestibility (Cervantes-Pahm and Stein, 2010; Goebel and Stein, 2011).

Early work conducted by Zhu et al. (1998) suggested that ESBM (HP 300) could replace dried whey, fish meal, full fatted extruded soybeans, and a portion of conventional SBM without negatively influencing performance. Yang et al. (2007) evaluated the efficacy of five soy protein sources (SBM, soy protein concentrate, ESBM (HP 300), and two fermented soy protein products) included at 8% of the diet for 14-d on weaned pig growth performance. Results indicated that replacing SBM on a kg/kg basis with ESBM increased ADG, ADFI and G:F. More recently, Jeong and Kim (2015) evaluated the efficacy of replacing 50% of fish meal (5 and 3% in phase 1 and 2, respectively) on a kg/kg basis with FSBM or ESBM (HP 300). The authors reported that ADG, G:F, and BW were similar for pigs fed fish meal and ESBM in phase 1, 2, and overall (d 0 to 42). However, pigs fed fish meal had increased ADFI compared to pigs fed ESBM for all phases and overall. These findings are in agreement with the current studies, where increasing ESBM at the expense of SBM and/or fish meal resulted in decreased ADFI and final

BW. The reduction in ADFI observed in both studies as pigs were fed increasing levels of ESBM may have been the result of its greater water holding capacity compared to the other protein sources it replaced. Kyriazkis and Emmans (1995) observed that an increase in water holding capacity resulted in a reduction of feed intake due to greater gut fill. Analysis of SBM, fish meal, and ESBM used in Exp. 1 indicated that ESBM had the greatest water holding capacity. Thus, the increase in ESBM at the expense of SBM and fish meal may have potentially resulted in greater gut fill because of its greater water holding capacity.

Another possible explanation for the poorer ADFI when pigs were fed increasing amounts of ESBM could be attributed to palatability. Previous preference work conducted by Solà-Oriol et al. (2011) demonstrated a linear reduction in preference to increasing inclusion rates of soybean meal and other soy proteins for young pigs. Furthermore, it was reported that intake preference to SBM or other soy peptides was inferior to animal-based proteins. These findings would agree with the reduction in feed intake observed in our studies when ESBM replaced fish meal. In addition, sensory testing in humans has indicated that soy products that have been modified by enzymes are perceived as bitter and producing an astringent taste (Cho et al., 2004). It is believed that the proteolytic enzymes expose the hydrophobic AA found in the interior portion of the protein, resulting in the bitter taste (Kurst, 2003). While diet preference or palatability was not directly evaluated in these studies, it has the potential to explain this reduced ADFI effect associated with high levels of ESBM.

Furthermore, it's of interest how performance of pigs fed 15% ESBM was significantly poorer in Exp. 2 than pigs fed ESBM replacing fish meal on a kg/kg basis. In addition, while not statistically significant, there were also noticeable numerical decreases in growth observed among pigs fed diets with (9%) ESBM replacing fish meal on a Lys basis compared to pigs fed

ESBM replacing fish meal on a kg/kg basis. This suggests inclusion level of ESBM plays an important role in growth performance. As previously demonstrated by Cervantes-Pahm and Stein (2010), the enzymatic treatment of soybean meal effectively reduced anti-nutritional factors which improved AA availability. However, Jeong and Kim (2015) suggested that the total amount of essential AA was still lacking in comparison to fish meal. Thus, it may be that these differences in the total amount of essential AA resulted in the poorer performance associated with higher inclusions of ESBM.

In conclusion, nursery pigs fed diets with 9% ESBM or greater resulted in poorer feed intake and final BW. While it is not entirely clear why higher levels of the ESBM tested in our trials elucidated this response, the observed results may be related to the increase in water holding capacity and/or a palatability issue.

LITERATURE CITED

AOAC International. 2006. Official Methods of Analysis of AOAC Int. 18th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

AOAC International. 2012. Official Methods of Analysis of AOAC Int. 19th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

Bergström, J. R., J. L. Nelssen, M. D. Tokach, R. D. Goodband, S. S. Dritz, K. Q. Owen, and W. B. Nessmith, Jr. 1997. Evaluation of spray-dried animal plasma and select menhaden fish meal in transition diets of weaned pigs at 12 to 14 days of age and reared in different production systems. J. Anim. Sci. 75:3004-3009. doi: 10.2527/1997.75113004x.

Cervantes-Pahm, S. K., and H. H. Stein. 2010. Ileal digestibility of amino acids in conventional, fermented, and enzyme-treated soybean meal and in soy protein isolate, fish meal, and casein fed to weanling pigs. J. Anim. Sci. 88:2674-2683. doi: 10.2527/jas.2009-2677.

Chen, F., Y. Hao, X. S. Piao, X. Ma, G. Y. Wu, S. Y. Qiao, D. F. Li, and J. J. Wang. 2011. Soybean-derived β-conglycinin affects proteome expression in pig intestinal cells in vivo and in vitro. J. Anim. Sci. 89:743-753. doi: 10.2527/jas.2010-3146.

Cho, M. J., N. Unklesbay, F. Hsih, A. D. Clark. 2004. Hydrophobicity of bitter peptides from soy protein hydrolysates. J. Agric. Food Chem. 52:5895-5901. doi:10.1021/jf0495035.

Cromwell, G. L. 2000. Utilization of soy products in swine diets. In: K. Drackley, editor, Soy in Animal Nutrition. Fed. Anim. Soc., Savoy, IL. p. 274-280.

Goebel, K. P., and H. H. Stein. 2011. Phosphorous and energy digestibility of conventional and enzyme treated soybean meal fed to weanling pigs. J. Anim. Sci. 89:764-772. doi: 10.2527/jas.2010-3253.

Jeong, J. S., and I. H. Kim. 2015. Comparative efficacy of up to 50% partial fish meal replacement with fermented soybean meal or enzymatically prepared soybean meal on growth performance, nutrient digestibility and fecal microflora in weaned pigs. Anim. Sci. J. 86:624-633. doi:10.1111/asj.12335.

Kim, S. W., and R. A. Easter. 2001. Nutritional value of fish meals in the diet for young pigs. J. Anim. Sci. 79:1829-1839. doi:/2001.7971829x.

Kurst, T. 2003. Protein modification to optimize functionality: Protein hydrolysates. In: R. Whitaker, A. G. J. Voragen, D. W. S. Wong, editors. Handbook of food enzymology. New York: Marcel Dekker, Inc. p. 221-253.

Kyrizakis, I., and G. C. Emmans. 1995. The voluntary feed intake of pigs given feeds based on wheat bran, dried citrus pulp, and grass meal, in relation to measurements of feed bulk. Brit. J. Nutr. 73:191-207. doi: 10.1079/BJN19950023.

Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, J. D. Hancock, G. L. Allee, R. D. Goodband, and R. D. Klemm. 1990. Transient hypersensitivity to soybean meal in the early-weaned pig. J Anim. Sci. 68:1790-1799. doi: 10.2527/1990.6861790x.

Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, R. Klemm, and R. D. Goodband. 1991. Interrelationship between hypersensitivity to soybean proteins and growth performance in early-weaned pigs. J. Anim. Sci. 69:4062-4069. doi: 10.2527/1991.69104062x.

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Solà-Oriol, D., E. Roura, and D. Torrallardona. 2011. Preference in pigs: effect of selected protein, fat, and fiber sources at different inclusion rates. J. Anim. Sci. 89:3219-3227. doi: 10.2527/jas.2011-3885.

Stoner, G. R., G. L. Allee, J. L. Nelssen, M. E. Johnston, and R. D. Goodband. 1990. Effect of select menhaden fishmeal in starter diets for pigs. J. Anim. Sci. 68:2729-2735. doi:/1990.6892729x.

Wiseman, J., S. Jagger D. J. A. Cole, and W. Haresign. 1991. The digestion and utilization of amino acids of heat-treated fish meal by growing/finishing pigs. Anim. Prod. 53:215-225. doi: 10.1017/S0003356100020158.

Yang, Y. X., Y. G. Kim, J. D. Lohakare, J. H. Yun, J. K. Lee, M. S. Kwon, J. I. Park, J. Y. Choi, and B. J. Chae. 2007. Comparative efficacy of different soy protein sources on growth performance, nutrient digestibility and intestinal morphology of weaned pigs. Asian-Aust. J. Anim. Sci. 20:775-783. doi:10.5713/ajas.2007.775.

Yen, J. T., A. H. Jensen, and J. Simon. 1977. Effect of dietary raw soybean and soybean trypsin inhibitor on trypsin and chymotrypsin activities in the pancreas and in small intestinal juice of growing swine. J. Nutr. 107:156-165.

Zhu, X., D. Li, S. Qiao, C. Xiao, Q. Qiao, and C. Ji. 1998. Evaluation of HP 300 soybean protein in starter pig diets. Asian-Aust. J. Anim. Sci. 11:201-207. doi: 10.5713/ajas.1998.201.

Table 3-1. Phase 1 diet composition, Exp. 1 (as-fed basis)[1,2]

		ESBM			ESBM
Ingredient, %	Control	Low	Medium	High	replacing fish meal
Corn	41.19	40.39	39.66	38.87	36.40
Soybean meal, 46.5% CP	19.35	15.62	11.88	8.15	19.35
Fish meal	7.50	5.00	2.50	---	---
ESBM[3]	---	6.67	13.33	20.00	10.21
Corn DDGS[4]	5.00	5.00	5.00	5.00	5.00
Whey permeate	18.75	18.75	18.75	18.75	18.75
Spray dried animal plasma	2.50	2.50	2.50	2.50	2.50
Tallow	3.00	3.00	3.00	3.00	3.95
Limestone	0.75	0.91	1.05	1.20	1.16
Monocalcium P, 21% P	0.25	0.52	0.74	1.00	1.08
Sodium chloride	0.25	0.25	0.25	0.25	0.25
L-Lys HCl	0.33	0.33	0.33	0.33	0.33
DL-Met	0.15	0.15	0.16	0.16	0.17
L-Thr	0.16	0.14	0.12	0.11	0.13
L-Trp	0.04	0.03	0.01	---	0.01
L-Val	0.11	0.07	0.04	---	0.05
Phytase[5]	0.04	0.04	0.04	0.0	0.04
Zinc oxide	0.42	0.42	0.42	0.42	0.42
Trace mineral premix[6]	0.13	0.13	0.13	0.13	0.13
Vitamin premix[7]	0.10	0.10	0.10	0.10	0.10
TOTAL	100	100	100	100	100
Calculated analysis					
Standardized ileal digestible (SID) amino acids, %					
Lys	1.35	1.35	1.35	1.35	1.35
Ile:Lys	55	58	60	63	61
Leu:Lys	117	119	122	125	122
Met:Lys	36	35	35	34	34
Met & Cys:Lys	57	57	57	57	57
Thr:Lys	63	63	63	63	63
Trp:Lys	20	20	20	20	20
Val:Lys	73	73	73	73	73
ME, kcal/kg	3,519	3,525	3,541	3,541	3,538
NE, kcal/kg	2,643	2,641	2,641	2,639	2,643
CP, %	21.9	22.1	22.4	22.6	22.3
Ca, %	0.78	0.78	0.78	0.78	0.78
P, %	0.64	0.65	0.65	0.66	0.67
Available, P%	0.56	0.56	0.56	0.56	0.56

[1]Phase 1 diets were fed from 5.1 to approximately 5.4 kg.

[2]Omega Special Select Menhaden (Omega Protein, Houston, TX).

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

[4]Dried distillers grains with solubles.

[5]Quantum Blue (AB-Vista Americas, Plantation, FL) provided 2,000 phytase units (FTU/kg) of diet with a release of 0.14% available P.

[6]Provided per kg of premix: 26.5 g Mn from manganese oxide; 110 g Fe from iron sulfate; 110 g Zn from zinc sulfate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[7]Provided per kg of premix: 4,509,410 IU vitamin A; 701,464 IU vitamin D3; 24,050 IU vitamin E; 3,007 mg riboflavin; 1,764 mg menadione; 12,025 mg pantothenic acid; 33,069 mg niacin; 15 mg vitamin B12.

Table 3-2. Phase 2 and 3 diet composition, Exp. 1 (as-fed basis)[1,2]

	Phase 2					Phase 3
		ESBM			ESBM	Common
	Control	Low	Medium	High	replacing fish meal	Diet
Corn	53.53	52.98	52.41	51.80	49.98	56.48
Soybean meal, 46.5% CP	22.22	19.42	16.62	13.82	22.22	27.94
Fish meal[3]	5.63	3.75	1.87	---	---	---
ESBM[4]	---	5.00	10.00	15.00	7.65	---
Corn DDGS[5]	5.00	5.00	5.00	5.00	5.00	10.00
Whey permeate	8.75	8.75	8.75	8.75	8.75	---
Tallow	2.00	2.00	2.00	2.00	2.70	2.00
Limestone	0.78	0.90	1.00	1.13	1.08	1.03
Monocalcium P, 21% P	0.55	0.70	0.90	1.10	1.15	1.10
Sodium chloride	0.36	0.36	0.36	0.36	0.36	0.40
L-Lys HCl	0.45	0.45	0.45	0.45	0.45	0.45
DL-Met	0.16	0.17	0.17	0.17	0.18	0.20
L-Thr	0.19	0.18	0.16	0.15	0.16	0.15
L-Trp	0.06	0.05	0.04	0.03	0.04	0.01
L-Val	0.08	0.05	0.03	---	0.03	---
Phytase[6]	0.03	0.03	0.03	0.03	0.03	0.03
Trace mineral premix[7]	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix[8]	0.13	0.13	0.13	0.13	0.13	0.13
TOTAL	100	100	100	100	100	100
Calculated analysis						
Standardized ileal digestible (SID) amino acids, %						
Lys	1.30	1.30	1.30	1.30	1.30	1.25
Ile:Lys	55	57	59	61	59	59
Leu:Lys	117	119	121	123	121	131
Met:Lys	37	37	36	36	36	40
Met & Cys:Lys	57	57	57	57	57	62
Thr:Lys	63	63	63	63	63	63
Trp:Lys	20	20	20	20	20	17

Val:Lys	68	68	68	68	68	66
ME, kcal/kg	3,433	3,439	3,446	3,450	3,450	3,371
NE, kcal/kg	2,571	2,568	2,568	2,568	2,571	2,493
CP, %	21.0	21.2	21.3	21.5	21.3	21.4
Ca, %	0.74	0.74	0.74	0.74	0.74	0.69
P, %	0.64	0.64	0.64	0.65	0.65	0.64
Available, P %	0.51	0.51	0.51	0.51	0.51	0.48

[1]Phase 2 diets were fed from 5.4 to approximately 9.8 kg. Phase 3 diets were fed from approximately 9.8 kg to approximately 20.6 kg.

[2]The high ESBM treatment was accomplished by including HP300 at the expense of soybean meal (SBM) and fish meal (fish meal). The fish meal control diet and the HP 300 (high inclusion) replacing soybean meal and fish meal were blended to form the intermediate diets in phase 2.

[3]Omega Special Select Menhaden (Omega Protein, Houston, TX).

[4]Enzymatically-treated soybean meal (HP 300; Hamlet Protein, Findlay, OH).

[5]Dried distillers grain with solubles.

[6]Optiphos 2000 (Huvepharma, Inc., Sofia, Bulgaria) provided 1,254.4 phytase units (FTU/kg) of diet with a release of 0.13% available P.

[7]Provided per kg of premix: 26.5 g Mn from manganese oxide; 110 g Fe from iron sulfate; 110 g Zn from zinc sulfate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[8]Provided per kg of premix: 4,509,410 IU vitamin A; 701,464 IU vitamin D3; 24,050 IU vitamin E; 3,007 mg riboflavin; 1,764 mg menadione; 12,025 mg pantothenic acid; 33,069 mg niacin; 15 mg vitamin B12.

Table 3-3. Chemical analysis of ESBM and Menhaden fish meal, Exp. 2 (as-fed basis)[1,2]

Item	ESBM[3]	Menhaden fish meal[4]
Proximate analysis, %		
DM	93.94	93.01
CP	55.74	63.25
Ca	0.40	5.17
P	0.67	2.61
Ether extract	1.55	10.70
Ash	6.31	19.11
Total AA, %		
Arg	3.88	3.69
Cys	0.76	0.48
His	1.40	1.51
Ile	2.71	2.52
Leu	4.36	4.28
Lys	3.29	4.82
Met	0.76	1.68
Phe	2.84	2.40
Thr	2.11	2.40
Trp	0.81	0.61
Tyr	1.96	1.79
Val	2.86	3.09

[1]Proximate analysis by Ward Laboratories Inc., (Kearney, NE).

[2]Amino acid analysis by the University of Missouri-Columbia College of Agriculture, Food and Natural Resources – Agriculture Experiment Station Chemical Laboratories (Columbia, MO).

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

[4]LT Prime Menhaden Fishmeal (Daybrook Fisheries, Inc., New Orleans, LA).

Table 3-4. Diet composition, Exp. 2 (as-fed basis)[1]

Ingredient, %	Negative control	Fish meal control	ESBM replacing fish meal		15% ESBM diet
			SID Lys. basis	kg for kg	
Corn	40.29	48.33	43.51	46.53	45.71
Soybean meal, 46.5% CP	32.77	21.35	21.35	21.35	13.82
Corn DDGS[2]	10.00	10.00	10.00	10.00	10.00
Spray-dried whey	10.00	10.00	10.00	10.00	10.00
Fish meal[3]	---	6.00	---	---	---
ESBM[4]	---	---	9.10	6.00	15.00
Choice white grease	3.00	1.25	2.10	1.85	1.50
Limestone	1.07	0.62	1.10	1.10	1.13
Monocalcium P, 21% P	1.05	0.45	1.00	1.05	0.98
Sodium chloride	0.50	0.50	0.50	0.50	0.50
L-Lys HCl	0.35	0.39	0.39	0.50	0.43
DL-Met	0.15	0.16	0.15	0.19	0.16
L-Thr	0.11	0.17	0.12	0.17	0.12
L-Trp	---	0.04	---	0.02	---
L-Val	0.05	0.09	0.02	0.09	---
Phytase[5]	0.02	0.02	0.02	0.02	0.02
Zinc oxide	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[6]	0.15	0.15	0.15	0.15	0.15
Vitamin premix[7]	0.25	0.25	0.25	0.25	0.25
TOTAL	100	100	100	100	100
Calculated analysis					
Standardized ileal digestible (SID) amino acids, %					
Lys	1.35	1.35	1.35	1.35	1.35
Ile:Lys	64	58	64	59	65
Leu:Lys	131	124	132	125	132
Met:Lys	35	38	35	36	36
Met & Cys:Lys	58	58	58	58	58
Thr:Lys	63	63	63	63	63
Trp:Lys	18.7	18.7	18.7	18.7	18.7
Val:Lys	72	72	72	72	72
Total Lysine, %	1.52	1.53	1.53	1.52	1.53
ME, kcal/kg	3,402	3,377	3,402	3,382	3,399
NE, kcal/kg	2,502	2,502	2,502	2,502	2,502
CP, %	23.4	22.6	23.3	22.0	23.2
Ca, %	0.78	0.78	0.78	0.78	0.78
P, %	0.69	0.66	0.67	0.67	0.66
Available P, %	0.51	0.51	0.51	0.51	0.51

[1]Diets were fed from 6.2 to approximately 11.8 kg.

[2]Dried distillers grain with solubles.

[3]LT Prime Menhaden Fishmeal (Daybrook Fisheries, Inc., New Orleans, LA).

[4]Enzymatically-treated soybean meal (HP 300; Hamlet Protein, Findlay, OH).

[5]Ronozyme HiPhos 2700 (DSM Nutritional Products, Parsippany, NJ) provided 476 phytase units (FTU/kg) of diet with a release of 0.10% available P.

[6]Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulphate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[7]Provided per kilogram of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

Table 3-5. Chemical analysis of phase 1 and phase 2 diets, Exp. 1 (as-fed basis)[1,2]

Item, %	Control	ESBM			ESBM replacing fish meal
		Low	Medium	High	
Phase 1 diets					
DM[3]	90.35	90.47	91.17	90.58	90.7
CP[3]	21.4	22.0	21.7	22.7	22.7
Ca[3]	1.34	1.06	0.91	0.96	0.96
P[3]	0.74	0.72	0.69	0.75	0.73
Ether extract[3]	5.4	5.6	5.4	5.0	5.7
Water holding capacity, g H_2O/g feed[4]	1.75	2.06	2.13	2.41	2.21
Total AA[5], %					
Arg	1.17 (1.28)	1.23 (1.31)	1.28 (1.34)	1.32 (1.38)	1.38 (1.37)
His	0.50 (0.55)	0.50 (0.57)	0.52 (0.58)	0.54 (0.59)	0.55 (0.58)
Ile	0.86 (0.87)	0.88 (0.90)	0.91 (0.93)	0.94 (0.96)	0.94 (0.93)
Leu	1.72 (1.82)	1.77 (1.85)	1.81 (1.88)	1.85 (1.92)	1.85 (1.88)
Lys	1.37 (1.53)	1.33 (1.53)	1.34 (1.54)	1.40 (1.54)	1.41 (1.53)
Met	0.43 (0.54)	0.45 (0.52)	0.43 (0.51)	0.42 (0.49)	0.43 (0.49)
Met + Cys	0.76 (0.89)	0.81 (0.88)	0.79 (0.87)	0.80 (0.86)	0.83 (0.87)
Thr	0.91 (1.01)	0.94 (1.00)	0.94 (0.99)	0.95 (0.98)	0.98 (0.99)
Trp	0.27 (0.31)	0.28 (0.30)	0.27 (0.30)	0.27 (0.30)	0.28 (0.30)
Val	1.09 (1.15)	1.09 (1.13)	1.07 (1.12)	1.07 (1.11)	1.10 (1.12)
Phe	0.96 (0.98)	0.99 (1.02)	1.03 (1.05)	1.07 (1.09)	1.11 (1.07)
Free Lys	0.21 (0.33)	0.22 (0.33)	0.21 (0.33)	0.22 (0.33)	0.22 (0.33)
Phase 2 diets					
DM[3]	87.02	88.96	88.65	87.16	88.6
CP[3]	21.2	21.3	21.8	22.3	21.2
Ca[3]	1.02	0.98	1.04	0.95	1.01
P[3]	0.65	0.62	0.67	0.68	0.69
Ether extract[3]	5.1	4.8	4.7	4.4	4.8
Water holding capacity g H_2O/g feed[4]	1.49	1.46	1.78	1.83	1.61
Flowdex (mm)[6]	30	30	30	30	28
Angle of repose	40.7	41.7	41.2	41.2	41.7
Total AA[5], %					
Arg	1.24 (1.24)	1.27 (1.27)	1.29 (1.29)	1.30 (1.31)	1.25 (1.31)
His	0.52 (0.53)	0.53 (0.54)	0.54 (0.55)	0.54 (0.55)	0.52 (0.55)
Ile	0.88 (0.83)	0.90 (0.86)	0.92 (0.88)	0.94 (0.90)	0.89 (0.88)
Leu	1.74 (1.75)	1.78 (1.78)	1.79 (1.80)	1.82 (1.82)	1.71 (1.80)
Lys	1.50 (1.46)	1.49 (1.46)	1.47 (1.47)	1.45 (1.47)	1.36 (1.46)
Met	0.49 (0.53)	0.47 (0.52)	0.47 (0.51)	0.44 (0.50)	0.44 (0.50)
Met + Cys	0.79 (0.85)	0.80 (0.84)	0.81 (0.84)	0.78 (0.83)	0.77 (0.83)
Thr	0.94 (0.96)	0.93 (0.95)	0.96 (0.94)	0.89 (0.93)	0.87 (0.94)
Trp	0.28 (0.30)	0.28 (0.29)	0.25 (0.29)	0.28 (0.29)	0.26 (0.29)

Val	1.06 (1.03)	1.04 (1.02)	1.02 (1.01)	1.00 (1.01)	0.98 (1.01)
Phe	0.97 (0.95)	1.01 (0.98)	1.02 (1.00)	1.04 (1.03)	0.99 (1.01)
Free Lys	0.33 (0.45)	0.28 (0.45)	0.27 (0.45)	0.28 (0.45)	0.24 (0.45)

[1]Complete diet samples were obtained from each dietary treatment each week during the study and composited.

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

[3]Composite samples were submitted to Ward Laboratories (Kearney, NE) for analysis.

[4]Water holding capacity.

[5]Amino acid analysis of complete diets were analyzed in duplicate by Ajinomoto Heartland, Inc. (Chicago, IL). Values in parenthesis indicate formulated values.

[6]Flowdex (Hanson Research, Chatsworth, CA) – flowability index represents the smallest diameter disk in which 50 grams of sample flows through on three consecutive attempts.

Table 3-6. Chemical analysis of phase 3 common diet, Exp. 1 (as-fed basis)[1,2]

Item, %	Common diet
DM	87.86
CP	18.7
Ca	0.74
P	0.60
Ether extract	5.0

[1]Complete diet samples were obtained from each dietary treatment each week during the study and composited.

[2]Composite samples were submitted to Ward Laboratories (Kearney, NE) for analysis.

Table 3-7. Chemical analysis of diets, Exp. 2 (as-fed basis)[1,2,3]

Item	Negative control	Fish meal control	ESBM replacing fish meal		ESBM diet
			SID Lys basis	kg for kg	
DM	90.98	91.11	91.13	91.29	91.98
CP	23.2	22.6	23.9	22.6	24.00
Ca	1.02	0.89	1.02	1.00	0.96
P	0.76	0.67	0.68	0.72	0.73
Ether extract	6.2	5.1	5.2	5.1	4.8

[1]Complete diet samples were obtained from each dietary treatment each week during the study and composited.

[2]Composite samples were submitted to Ward Laboratories (Kearney, NE) for analysis.

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

Table 3-8. Effects of feeding increasing enzymatically treated soybean meal (ESBM) on nursery pig performance, Exp. 1[1]

							Probability, $P <$		
		ESBM[2]			ESBM replacing fish meal	SEM	ESBM		Control vs. ESBM replacing fish meal
	Control	Low	Medium	High			Linear	Quadratic	
BW, kg									
d 0	5.11	5.10	5.10	5.10	5.10	0.074	0.975	0.944	0.958
d 7	5.42	5.33	5.44	5.46	5.42	0.076	0.183	0.169	0.998
d 22	10.08	9.75	9.61	9.57	9.82	0.182	0.008	0.273	0.175
d 43	20.98	20.60	20.41	20.29	20.54	0.289	0.045	0.595	0.209
d 0 to 7									
ADG, g	45	32	48	50	44	5.1	0.134	0.122	0.950
ADFI, g	200	193	187	204	198	4.2	0.773	0.001	0.696
G:F	0.222	0.165	0.253	0.247	0.221	0.0239	0.123	0.289	0.975
d 7 to 22									
ADG, g	302	284	272	269	285	8.9	0.002	0.316	0.118
ADFI, g	401	394	379	366	392	7.8	<0.001	0.636	0.362
G:F	0.749	0.719	0.713	0.731	0.727	0.0133	0.299	0.061	0.206
d 0 to 22									
ADG, g	219	202	200	199	207	6.7	0.020	0.157	0.135
ADFI, g	336	328	317	314	329	5.5	0.001	0.606	0.276
G:F	0.643	0.609	0.622	0.625	0.624	0.0126	0.406	0.110	0.226
Common diet (d 22 to 43)									
ADG, g	516	516	508	508	507	9.6	0.426	0.942	0.474
ADFI, g	740	724	722	720	719	14.4	0.298	0.580	0.268
G:F	0.697	0.713	0.705	0.705	0.705	0.0046	0.441	0.073	0.235
d 0 to 43									
ADG, g	361	353	348	349	351	6.4	0.120	0.444	0.248
ADFI, g	530	518	511	511	517	8.2	0.071	0.460	0.243
G:F	0.680	0.681	0.680	0.681	0.679	0.0034	0.963	0.929	0.845

[1]A total of 1,215 pigs (327 × 1050, PIC, Hendersonville, TN; initial BW 5.10 kg) with 27 pigs per pen and 9 replications per treatment were used in a 43-d growth trial. Experimental diets were fed in 2 phases (d 0 to 7 and 7 to 22) with a common diet fed from d 22 to 43.

[2]Enzymatically-treated soybean meal (HP 300; Hamlet Protein, Findlay, OH). Low (ESBM: 6.67% and 5% included in phase 1 and 2, respectively), Med. (ESBM: 13.33% and 10% included in phase 1 and 2, respectively), High (ESBM: 20% and 15% included in phase 1 and 2, respectively).

Table 3-9. Effects of replacing fish meal with enzymatically treated soybean meal (ESBM) on nursery pig performance, Exp. 2[1,2]

	Negative control	Fish meal[3] control	ESBM replacing fish meal		15% ESBM diet	SEM	Probability, *P* <
			SID Lys basis	kg for kg			
BW, kg							
d 0	6.19	6.19	6.19	6.19	6.19	---	1.000
d 21	11.54^{b}	12.30^{a}	11.64^{b}	12.12^{ab}	11.48^{b}	0.238	0.042
d 0 to 21							
ADG, g	247^{yz}	278^{x}	253^{yz}	269^{xy}	245^{z}	10.2	0.080
ADFI, g	382^{ab}	407^{a}	354^{bc}	379^{abc}	352^{c}	11.1	0.003
G:F	0.649^{b}	0.687^{a}	0.713^{a}	0.709^{a}	0.693^{a}	0.0115	0.002

^{abc} Means within the same row with different superscripts differ ($P < 0.05$).

^{xyz} Means within the same row with different superscripts differ ($P < 0.10$).

[1]A total of 350 barrows (Line 200 × 400 DNA, Columbus, NE; initially 6.2 kg) with 5 pigs per pen and 14 replications per treatment were used in a 21-d growth performance trial

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

[3]LT Prime Menhaden Fishmeal (Daybrook Fisheries, Inc., New Orleans, LA.

Chapter 4 - Evaluating of dietary electrolyte balance on nursery pig performance

ABSTRACT

A total of 2,880 pigs (327 × L42 PIC, Hendersonville, TN; initially 5.2 kg) were used to determine the effects of increasing dietary electrolyte balance (dEB) on nursery pig performance. There were 30 pigs per pen (60 pigs per double-sided feeder) and 12 replications (feeder) per treatment. Pens of pigs were allotted by BW and gender on arrival, and randomly assigned to 1 of 4 dietary treatments. Dietary treatments were corn-soybean meal-based with spray-dried whey and other specialty protein sources used from d 0 to 21. Dietary electrolyte balance was determined using the following equation: dEB = ((Na*434.98) + (K*255.74) – (Cl*282.06)) mEq/ kg. From d 0 to 8, diets had dEB's of 84, 137, 190, and 243 mEq/kg. During d 8 to 21, diets had dEB's of 29, 86, 143, and 199 mEq/kg. After d 21 of experimental diets, a common diet was fed from d 21 to 35 to all pigs and was a typical nursery diet fed in commercial production with a dEB of 257 mEq/kg. During d 0 to 8, increasing dEB increased (quadratic, $P < 0.05$) ADG, ADFI, G:F and d 8 BW. From d 8 to 21, increasing dEB improved (quadratic, $P = 0.022$) ADG and ADFI (linear, $P = 0.001$) as dEB was increased, resulting in an improvement in G:F (quadratic, $P = 0.001$). During d 0 to 21, increasing dEB increased (linear, $P < 0.05$) ADG, ADFI, and d 21 BW, and improved (quadratic, $P < 0.001$) G:F. During d 21 to 35 (common period), pigs that were previously fed low dEB diets had increased (linear, $P < 0.001$) ADG and marginally improved (quadratic, $P = 0.091$) G:F; however, no evidence for differences were detected for ADFI. For the overall nursery period (d 0 to 35), increasing dEB from d 0 to 21 increased (linear, $P < 0.001$) ADG and final BW, which was the result of increased (quadratic, *P*

< 0.001) G:F and marginally greater (linear, $P = 0.077$) ADFI. In conclusion, increasing dEB up to 243 and 199 mEq/kg of diet in phase 1 and 2, respectively in nursery diets improved growth performance of weanling pigs.

Key words: chloride**,** dietary electrolyte balance, growth performance, nursery pig

INTRODUCTION

Electrolytes are key minerals that can be defined as chemical substances that separate when dissolved in fluids to form positive (cation) and negative (anion) ions. These charged ions produce an electrically conductive current that serves as a medium for cellular signaling, biochemical reactions, transport of substrates across cellular membranes, and the removal of waste products from the body among others. Previous research has indicated that cations and anions are closely linked to the alkalinity and acidity of body fluids (Mustaq and Pasha, 2013). In particular, the monovalent minerals Na, K, and Cl are considered strong ions due to their ability to exert significant shifts in acid-base homeostasis (Mongin, 1981).

Commonly, ingredients such as calcium carbonate, calcium phosphate, and sodium chloride are included in swine diets to meet mineral requirements, but they also contribute to the dietary electrolyte balance (dEB), thus potentially altering acid-base homeostasis and growth performance of pigs (Patience et al., 1987; Haydon et al., 1990; DeRouchey et al., 2003). Traditionally, the optimal dEB for pigs is reported to be approximately 250 mEq/kg (NRC, 2012), but limited research exists in this area. Recently, Guzmán-Pino et al. (2015) reported that nursery pigs had poorer ADG and G:F when dEB exceeded 150 mEq/kg. These researchers used $CaCl_2$ to lower dEB and reported 48.7% improvement in ADG by decreasing dEB from 269 to 16 mEq/kg. However, these results are contrary to other studies that have demonstrated improvements in growth performance with increasing dEB (Patience et al., 1987; Dersjent-Li

etal., 2001; DeRouchey et al., 2003). Thus, the objective of our study was to further investigate the influence of dEB on growth performance of nursery pigs.

MATERIALS AND METHODS

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The experiment was conducted at a commercial nursery in southeast MN. Pigs were housed in pens (1.82 × 3.35 m) that were equipped with a double sided, 5-hole stainless steel dry feeder and one cup waterer for ad libitum access to feed and water. The facility was equipped with a computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN) that delivered and recorded daily feed additions and diets as specified. This system is capable of feeding each individual pen any of the individual diets. Diets were manufactured from Hubbard Feeds (Mankato, MN) from d 0 to 8 and Bixby Feed Mill (Blooming Prairie, MN) from d 8 to 35.

Experimental Design

A total of 2,880 pigs (327 × L42 PIC, Hendersonville, TN; initially 5.2 kg) were used in a 35-d growth trial with 30 pigs per pen (60 pigs per double-sided feeder) and 12 replications (feeder) per treatment. Pens of pigs were allotted by BW and gender on arrival to the nursery, and randomly assigned to 1 of 4 dietary treatments. The treatments were corn-soybean meal-based with specialty protein sources used from d 0 to 8 (Table 4-1) with decreased amounts of the specialty ingredients during d 8 to 21 (Table 4-2). Diets 1 to 4 were formulated to contain increasing levels of dEB ranging from 84 to 243 mEq/kg during d 0 to 8 and 29 to 199 mEq/kg from d 8 to 21, respectively. The lowest dEB diets were achieved by adding 1.17% and 1.25% $CaCl_2$ from d 0 to 8 and 8 to 21, respectively. Dietary Ca concentrations were maintained in the

three highest dEB diets, but increased in the low dEB diet with the increasing level of $CaCl_2$. The following equation derived by Mongin (1981): dEB = ((Na*434.98) + (K*255.74) – (Cl*282.06)) mEq/ kg was used to determine dEB. After d 21 of experimental diets, a common diet was fed from d 21 to 35 (Table 4-3) to all pigs and was a typical nursery diet fed in commercial production with a dEB of 257 mEq/kg. From d 0 to 8, diets were fed in pellet form, while d 8 to 21 and d 21 to 35 were fed in meal form. The NRC (2012) nutrient and SID AA coefficients for ingredients were used in formulating diets. Pigs and feeders were weighed on d 0, 8, 15, 21, and 35 of the trial to determine ADG, ADFI, and G:F

Diet Sampling and Analysis

Complete diet samples were obtained from feeders, composited, and frozen at -20ºC for subsequent analysis. Composite samples of diets were split using a riffle splitter (Humboldt Mfg. Co., Norridge, IL) and processed through a 1 mm screen in a Willey mill (Thomas Scientific, Swedesboro, NJ) prior to analysis. All diet samples were submitted for analysis of DM (method 935.29; AOAC International, 2012), CP (method 990.03; AOAC International., 2012), ether extract (method 920.39; AOAC International, 2012) for preparation and analyzed using an ANKOM XT20 Fat Analyzer (Ankom Technology, Fairport, NY), Ca, P, and K (method 968.08; AOAC International, 2012) for preparation using ICAP 6500 (ThermoElectron Corp., Waltham, MA), Na (method 990.08; AOAC International, 2012), and Cl (method 969.10; AOAC International, 2012; Ward Laboratories Inc., Kearney, NE).

Statistical Analysis

Data were analyzed using the PROC GLIMMIX procedure in SAS (SAS Institute, Inc., Cary, NC) with feeder (two pens of 60 pigs) as the experimental unit, dietary treatment as a fixed effect, and block and room serving as the random effect in the model. Preplanned linear and

quadratic polynomial contrasts were used to determine the effects of increasing dEB. Results were considered significant at $P \leq 0.05$ and marginal effects between $P > 0.05$ and $P \leq 0.10$.

RESULTS

Chemical Analysis

Analysis of experimental diets showed that most nutrients were similar to formulated values for phase 1 diets (Table 4-4). Analyzed values for Na, K, and Cl were higher across dietary treatments in phase 2 (Table 4-5) than formulated values; however, the range of dEB targeted was ultimately maintained across dietary treatments in both d 0 to 8 and 8 to 21 diets.

From d 0 to 8, increasing dEB increased (quadratic, $P < 0.05$; Table 4-6) ADG, ADFI, G:F, and d 8 BW. From d 8 to 21, increasing dEB improved (quadratic, $P = 0.022$) ADG and ADFI (linear, $P = 0.001$) as dEB was increased, resulting in an improvement in G:F (quadratic, $P = 0.001$). During d 0 to 21, increasing dEB increased (linear, $P < 0.05$) ADG, ADFI, and d 21 BW, and improved G:F (quadratic, $P < 0.001$). During d 21 to 35 (common period), pigs that were previously fed low dEB diets had increased (linear, $P < 0.001$) ADG and marginally improved (quadratic, $P = 0.091$) G:F; however, no evidence for differences were detected for ADFI. For the overall nursery period (d 0 to 35), increasing dEB from d 0 to 21 increased (linear, $P < 0.001$) ADG and final BW, which was the result of increased (quadratic, $P = 0.030$) G:F and marginally greater (linear, $P = 0.077$) ADFI.

DISCUSSION

For the current experiment, dEB was determined by examining the interrelationship between the monovalent micromineral ions Na, K, and Cl: dEB = ((Na*434.98) + (K*255.74) – (Cl*282.06)) mEq/ kg (Mongin, 1981). Previous literature examining the effects of dEB on pigs have generally demonstrated improvements in growth performance when dEB was increased

with the NRC (2012) reporting the optimal electrolyte balance for pigs to be approximately 250 mEq/kg. However, there are discrepancies within the literature in regards to an optimal dEB range for growing pigs. Patience et al. (1987) conducted an experiment in which 6 levels (-85, 0, 100, 175, 277, and 341 mEq/kg) of dEB were fed to growing pigs (initially ~15 kg) for 28-d with the supplemental salts calcium chloride included in the three low dEB diets and sodium bicarbonate used in the three high diets. The authors reported that performance was optimized when pigs were fed a diet with a dEB of 175 mEq/kg. Similarly, Dersjent-Li et al. (2001) conducted an experiment in which pigs (initially ~9 kg) were fed 3 dEB concentrations (-100, 200, and 500 mEq/kg) by including $CaCl_2$ in the low dEB diet and $NaHCO_3$ in the other two treatments. They found that performance was optimized when pigs were fed a diet with a dEB between 200 and 500 mEq/kg. In contrast, Patience and Chaplin (1997) compared diets containing -20, 104, and 163 mEq/kg of dEB fed to pigs (initially ~35 kg). For this study, the supplemental salts $CaCl_2$, $NaHCO_3$, and $KHCO_3$ were included to alter dEB. Results indicated a tendency for improved growth when pigs were fed a dEB diet of -20 mEq/kg compared to pigs fed either 104 or 163 mEq/kg. However, the observed tendency for improved growth might have been a result of feed intake, as it was equalized across all treatments by feeding an amount that was equal to the pigs with the lowest feed intake as opposed to ad libitum feed intake allowed in other studies. Recently, Guzmán-Pino et al. (2015) conducted an experiment to determine the influence of dEB on growth performance of nursery pigs (initially ~13 kg). In their study, 4 dEB concentrations (16, 133, 152, and 269 mEq/kg) were fed to pigs from 21 to 37 d post-weaning with dEB altered by including $CaCl_2$ and $NaHCO_3$, respectively. The authors observed that increasing dEB decreased ADG and G:F when dEB exceeded 150 mEq/kg with a 48.7% improvement in ADG by decreasing dEB from 269 to 16 mEq/kg.

In the study herein, decreasing dEB in nursery diets resulted in a reduction in ADG and final BW, which was the result of marginally lower ADFI and poorer feed efficiency. A possible explanation for the lower feed intake in pigs fed the low dEB diet could be contributed to the $CaCl_2$ used to lower dEB. Yen et al. (1981) indicated that dietary $CaCl_2$ limited intake in pigs through Cl-induced metabolic acidosis. Furthermore, sensory tests using humans has indicated calcium chloride itself is perceived as producing a bitter and metallic off-flavor (Lawless et al., 2003; 2004). In addition, previous preference work conducted by Guzmán-Pino et al. (2015) examining a low (-16 mEq/kg; with $CaCl_2$) and high (388 mEq/kg; without $CaCl_2$) dEB diet reported that pigs fed the low dEB diet had decreased preference as opposed to the high dEB diet. However, when similar diets were used in a growth performance trial, performance decreased when pigs were fed levels above 150 mEq/kg of the diet. Personal communication with the authors indicated that a similar unprotected $CaCl_2$ source and the same equation was used to calculate the dEB as in our study. In addition, diets fed by Guzmán-Pino et al. (2015) were not analyzed for Na, K, and Cl, thus, making it difficult to assess whether dEB concentrations were similar to estimated values. Nevertheless, the reasons that their preference trial results did not match their growth performance trial results is unclear.

Interestingly, in contrast to the results of Guzmán-Pino et al. (2015); Lei et al. (2017) reported that weanling pigs (initially ~8 kg) fed diets with increasing dEB (0, 83, 166, and 250 mEq/kg) had improved ADG and ADFI. It must be noted that the authors altered dEB with the addition of $CaCl_2$ or $NaHCO_3$. Furthermore, increasing dEB resulted in improvements in ATTD of DM and N in pigs fed the high dEB diets (166 and 250 mEq/kg) compared to the low dEB diet (0 mEq/kg). It was hypothesized that the improvement in performance when pigs were fed high dEB was the result of the improvement in ATTD of DM and N. While digestibility

measurements were not quantified in the current study, the improvement in feed efficiency as dEB increased could be indicative of an improvement in digestibility. We have no explanation why Guzmán-Pino et al. (2015) observed the opposite response to Lei et al. (2017) and our data.

In conclusion, the results from this study indicate that increasing dEB up to 243 and 199 mEq/kg of diet in phase 1 and 2, respectively from weaning to d 21 after weaning resulted in an increase in ADG and final BW, which was the result of a marginally significant improvement ADFI and feed efficiency.

LITERATURE CITED

AOAC International. 2012. Official Methods of Analysis of AOAC Int. 19th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

Dersjant-Li, Y., H. Schulze, J. W. Schrama, J. A. Verreth, and M. W. A. Verstegen. 2001. Feed intake, growth, digestibility of dry matter and nitrogen in young pigs as affected by dietary cation-anion difference and supplementation of xylanase. J. Anim. Physiol. Anim. Nutr. 85:101-109. doi: 10.1046/j.1439-0396.2001.00307.x.

DeRouchey, J. M., J. D. Hancock, R. H. Hines, K. R. Cummings, D. J. Lee, C. A. Maloney, D. W. Dean, J. S. Park, and H. Cao. 2003. Effects of dietary electrolyte balance on the chemistry of blood and urine in lactating sows and sow litter performance. J. Anim. Sci. 81:3067-3074. doi:10.2527/2003.81123067x.

Guzmán-Pino, S. A., D. Solà-Oriol, R. Davin, E. G. Manzanilla, and J. F. Pérez. 2015. Influence of dietary electrolyte balance on feed preference and growth performance of postweaned piglets. J. Anim. Sci. 93:2840-2848. doi:10.2527./jas2014-8380.

Haydon, K. D., J. W. West, and M. N. McCarter. 1990. Effect of dietary electrolyte balance on performance and blood parameters of growing-finishing swine fed in high ambient temperatures. J. Anim. Sci. 68:2400-2406. doi:10.2527/1990.6882400x.

Lawless, H. T., F. Rapacki, J. Horne, and A. Hayes. 2003. The taste of calcium and magnesium salts and anionic modifications. Food Qual. Prefer. 14:319-325. doi:10.1016/S0950-3293(02)00128-3.

Lawless, H. T., F. Rapacki, J. Horne, A. Hayes, and G. Wang. 2004. The taste of calcium chloride in mixtures with NaCl, sucrose, and citric acid. Food Qual. Prefer. 15:83-89: doi:10.1016/S0950-3293(03)00099-5.

Lei, X. J., J. Y. Chung, J. H. Park, and I. H. Kim. 2017. Evaluation of different dietary electrolyte balance in weanling pigs diets. Anim. Feed. Sci. Technol. 226:98-102. doi:10.1016/j.anifeedsci.2017.02.014.

Mongin, P. 1981. Recent advances in dietary anion-cation balance: applications in poultry. Proc. Nutr. Soc. 40:285-294.

Mushtaq, M. M. H and T. N. Pasha. 2013. Electrolytes, dietary electrolyte balance and salts in broilers: an updated review on acid-base balance, blood and carcass characteristics. World Poult. Sci. J. 69:833-851. doi: 10.1017/S0043933913000846.

NRC. 2012. Nutrient requirements of swine. 11th ed. Natl. Acad. Press, Washington, DC.

Patience, J. F., R. E. Austic, and R. D. Boyd. 1987. Effect of dietary electrolyte balance on growth and acid-base status in swine. J. Anim. Sci. 64:457-466. doi: 10.2527/jas1987.642457x.

Patience, J. F., and R. K. Chaplin. 1997. The relationship among dietary undetermined anion, acid-base balance, and nutrient metabolism in swine. J. Anim. Sci. 75:2445-2452.

Yen, J. T., W. G. Pond, and R. L. Prior. 1981. Calcium chloride as a regulator of feed intake and weight gain in pigs. J. Anim. Sci. 52:778-782.

Table 4-1. Diet composition, d 0 to 8 after weaning (as-fed basis)[1]

	Dietary electrolyte balance (mEq/kg)[2]			
Ingredient, %	84	137	190	243
Corn	38.58	39.00	39.14	39.24
Soybean meal, 46.5% CP	17.71	17.68	17.67	17.66
Corn DDGS[3]	5.00	5.00	5.00	5.00
Fish meal[4]	4.50	4.50	4.50	4.50
HP 300[5]	2.50	2.50	2.50	2.50
Spray dried whey	25.00	25.00	25.00	25.00
Choice white grease	3.00	3.00	3.00	3.00
Moncalcium P, 21% P	0.40	0.40	0.40	0.40
Limestone	---	---	0.26	0.55
Calcium chloride	1.17	0.78	0.39	---
Sodium chloride	0.30	0.30	0.30	0.30
L-Lys HCl	0.48	0.48	0.48	0.48
MHA[6]	0.29	0.29	0.29	0.29
L-Thr	0.20	0.20	0.20	0.20
L-Trp	0.05	0.05	0.05	0.05
L-Val	0.10	0.10	0.10	0.10
Choline chloride, 60%	0.04	0.04	0.04	0.04
Phytase[7]	0.04	0.04	0.04	0.04
Zinc oxide	0.39	0.39	0.39	0.39
Selenium premix, 0.06%	0.05	0.05	0.05	0.05
Vitamin and mineral premix[8]	0.28	0.28	0.28	0.28
TOTAL	100	100	100	100
Calculated analysis				
Standardized ileal digestible AA, %				
Lys	1.40	1.40	1.40	1.40
Ile:Lys	55	55	55	55
Leu:Lys	111	111	111	111
Met:Lys	40	40	40	40
Met & Cys:Lys	59	59	59	59
Thr:Lys	64	64	64	64
Trp:Lys	19	19	19	19
Val:Lys	67	67	67	67
ME, kcal/kg	3,461	3,474	3,479	3,483
CP, %	21.0	21.0	21.0	21.0
Na, %	0.39	0.39	0.39	0.39
Cl, %	1.34	1.16	0.97	0.78
K, %	1.14	1.14	1.14	1.14
Ca, %	0.84	0.73	0.73	0.73
P, %	0.67	0.67	0.67	0.67
Available P, %	0.59	0.59	0.59	0.59

[1]From d 0 to 8, diets were fed from approximately 5.2 kg to approximately 5.7 kg BW.

[2]Dietary electrolyte balance was calculated using the following equation: ((Na*434.98) + (K*255.74) – (Cl*282.06)).

[3]Dried distillers grains with solubles.

[4]Omega Special Select Menhaden Fish meal (Omega Protein, Houston, TX).

[5]Hamlet Protein (Findlay, OH).

[6]Novus International (Saint Charles, MO).

[7]Quantum Blue 5G (AB Vista Americas, Plantation, FL) provided 2,000 phytase units (FTU/kg) of diet with a release of 0.14% available P.

[8]Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulphate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; 198 mg Se from sodium selenite; 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

Table 4-2. Diet composition d 8 to 21 after weaning (as-fed basis)[1]

	Dietary electrolyte balance (mEq/kg)[2]			
Ingredient, %	29	86	142	199
Corn	46.92	47.16	47.28	47.41
Soybean meal, 46.5% CP	24.70	24.68	24.67	24.66
Corn DDGS[3]	15.00	15.00	15.00	15.00
Lactose	5.00	5.00	5.00	5.00
Fish meal[4]	3.75	3.75	3.75	3.75
Choice white grease	1.00	1.00	1.00	1.00
Dicalcium P, 18.5% P	0.63	0.63	0.63	0.63
Limestone	---	0.20	0.50	0.80
Calcium chloride	1.25	0.83	0.42	---
Sodium chloride	0.35	0.35	0.35	0.35
L-Lys HCl	0.40	0.40	0.40	0.40
L-Thr	0.13	0.13	0.13	0.13
L-Trp	0.03	0.03	0.03	0.03
Zinc oxide	0.25	0.25	0.25	0.25
Iron oxide	0.10	0.10	0.10	0.10
Antibiotic[5]	0.20	0.20	0.20	0.20
Vitamin and mineral premix[6]	0.30	0.30	0.30	0.30
TOTAL	100	100	100	100
Calculated analysis				
Standardized ileal digestible AA, %				
Lys	1.35	1.35	1.35	1.35
Ile:Lys	61	61	61	61
Leu:Lys	129	129	129	129
Met:Lys	31	31	31	31
Met & Cys:Lys	57	57	57	57
Thr:Lys	63	63	63	63
Trp:Lys	19	19	19	19
Val:Lys	69	69	69	69
ME, kcal/kg	3,131	3,139	3,142	3,146
CP, %	23.5	23.5	23.6	23.6
Na, %	0.22	0.22	0.22	0.22
Cl, %	0.99	0.79	0.59	0.39
K, %	0.84	0.84	0.84	0.84
Ca, %	0.83	0.79	0.79	0.79
P, %	0.65	0.65	0.65	0.65
Available P, %	0.36	0.36	0.36	0.36

[1]During d 8 to 21, diets were fed from approximately 5.7 kg to approximately 7.6 kg BW.

[2]Dietary electrolyte balance was calculated using the following equation: ((Na*434.98) + (K*255.74) – (Cl*282.06)).

[3]Dried distillers grains with solubles.

[4]Omega Special Select Menhaden Fish meal (Omega Protein, Houston, TX).

[5]Aureomycin (Zoetis Animal Health, Florham Park, NJ).

[6]Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulphate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; 198 mg Se from sodium selenite; 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

Table 4-3. Diet composition d 21 to 35 after weaning (as-fed basis)[1]

Ingredient, %	
Corn	38.33
Soybean meal, 46.5% CP	31.99
DDGS[2]	25.00
Choice white grease	1.00
Limestone	1.25
Dicalcium P, 18.5% P	1.03
Salt	0.50
L-Lys HCl	0.40
DL-Met	0.11
L-Thr	0.10
Vitamin and mineral premix[3]	0.30
TOTAL	100
Calculated analysis	
Standardized ileal digestible AA, %	
Lys	1.35
Met:Lys	35
Met & Cys:Lys	59
Thr:Lys	64
Trp:Lys	18
Val:Lys	74
ME, kcal/kg	3,278
CP, %	25.39
Na, %	0.29
Cl, %	0.47
K, %	1.03
Ca, %	0.83
P, %	0.66
Available P, %	0.37
dEB, mEq/kg[4]	257
Analyzed Composition, %	
DM	88.37
CP	22.48
Crude fat	5.90
Ca	0.82
P	0.64

[1]Phase 3 diets were fed from approximately 7.6 kg to approximately 15.8 kg BW.

[2]Dried distillers grain with solubles.

[3]Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulphate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; 198 mg Se from sodium selenite; 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

[4]Dietary electrolyte balance was determined by analyzing complete diets for Na, K, and Cl. Analyzed values were then used in the following equation to calculate dEB: ((Na*434.98) + (K*255.74) – (Cl*282.06)).

Table 4-4. Chemical analysis of phase 1 diets (as-fed basis)[1]

	Dietary electrolyte balance (dEB, mEq/kg)			
Item	84	137	190	243
DM, %	90.54	90.73	91.22	90.81
CP, %	20.95	20.85	21.10	20.95
Ether extract, %	4.60	4.80	4.70	4.70
Na, %	0.36	0.43	0.45	0.39
K, %	1.26	1.26	1.28	1.25
Cl, %	1.36	1.21	0.99	0.80
Ca, %	1.02	0.98	0.95	0.90
P, %	0.75	0.67	0.72	0.72
dEB, mEq/kg[2]	95	168	244	264

[1]Chemical analysis for complete diets was analyzed by Ward Laboratories, Inc. (Kearney, NE).

[2]Dietary electrolyte balance was determined by analyzing complete diets for Na, K, and Cl. Analyzed values were then used with the following equation to calculate dEB: ((Na*434.98) + (K*255.74) – (Cl*282.06)).

Table 4-5. Chemical analysis of phase 2 diets (as-fed basis)[1]

	Dietary electrolyte balance (dEB, mEq/kg)			
Item	29	86	142	199
DM, %	88.16	88.71	88.71	88.36
CP, %	21.00	23.15	23.55	21.35
Ether extract, %	5.20	5.10	5.30	5.20
Na, %	0.33	0.35	0.30	0.30
K, %	0.93	0.94	1.06	1.00
Cl, %	1.11	1.13	0.85	0.77
Ca, %	1.33	1.57	1.40	1.59
P, %	0.68	0.86	0.87	0.82
dEB, mEq/kg[2]	68	74	162	169

[1]Chemical analysis for complete diets was analyzed by Ward Laboratories, Inc. (Kearney, NE).

[2]Dietary electrolyte balance was determined by analyzing complete diets for Na, K, and Cl. Analyzed values were then used with the following equation to calculate dEB: ((Na*434.98) + (K*255.74) – (Cl*282.06)).

Table 4-6. Effects of increasing dietary electrolyte balance on nursery pig performance[1]

	mEq/kg[2]						
d 0 to 8:	84	137	190	243			
d 8 to 21:	29	86	142	199		Probability, *P* <	
d 21 to 35:			257		SEM	Linear	Quadratic
BW, kg							
d 0	5.19	5.19	5.20	5.17	0.053	0.753	0.517
d 8	5.62	5.60	5.70	5.80	0.043	0.001	0.038
d 21	9.40	9.78	10.01	10.21	0.084	0.001	0.180
d 35	15.56	15.75	15.94	16.02	0.119	0.001	0.547
d 0 to 8							
ADG, g	53	48	57	74	4.9	0.001	0.001
ADFI, g	85	83	82	96	3.7	0.008	0.004
G:F	0.614	0.556	0.691	0.768	0.0378	0.001	0.049
d 8 to 21							
ADG, g	282	314	322	335	5.4	0.001	0.022
ADFI, g	357	364	361	375	5.8	0.001	0.469
G:F	0.788	0.860	0.888	0.887	0.0093	0.001	0.001
d 0 to 21							
ADG, g	193	211	219	235	3.3	0.001	0.807
ADFI, g	252	256	253	268	3.8	0.003	0.103
G:F	0.771	0.831	0.869	0.874	0.0084	0.001	0.001
Common diet (d 21 to 35)							
ADG, g	440	424	423	415	4.7	0.001	0.376
ADFI, g	598	597	604	594	7.3	0.891	0.461
G:F	0.736	0.712	0.700	0.699	0.0067	0.001	0.091
d 0 to 35							
ADG, g	290	295	299	306	3.4	0.001	0.736
ADFI, g	388	390	391	397	4.7	0.077	0.594
G:F	0.756	0.781	0.797	0.802	0.0052	0.001	0.030

[1]A total of 2,880 pigs (PIC 327 × 1050; initial BW 5.2 kg) with 30 pigs per pen (60 pigs per feeder) and 12 replications (feeders) per treatment were used in a 35-d growth performance trial. All experimental diets were fed in two phases (d 0 to 8, and d 8 to 21) with a common diet fed from d 21 to 35.

[2]Dietary electrolyte balance was calculated using the following formula: ((Na*434.98) + (K*255.74) – (Cl*282.06)).

Chapter 5 - Assessment of sampling technique from feeders on copper, zinc, calcium, and phosphorous analysis

ABSTRACT

Treatments were arranged in a split-plot design with the whole-plot consisting of 1 of 6 concentrations of dietary Cu (27 to 147 mg/kg total Cu included in the diet) and the subplot using 1 of 2 sampling techniques (probe vs. hand grab). A total of 6 feeders per dietary treatment were sampled using a 1.6 m brass open handle probe (Seedburo Equipment Company, Des Plaines, IL), which contained 10 openings spaced approximately 5.1 cm apart was utilized for this study. The probe was inserted into the feeder on average 4 times to obtain ~ 900 g of sample. Alternatively, samples were simply collected by inserting a bare hand into the feeder approximately 8 times to obtain the ~900 g of sample. Within a feeder and sampling technique, subsamples (~200 g) were created by using a sample splitting device. Next, all samples were ground through a centrifugal mill and submitted for mineral analysis in duplicate. In addition to the 6 individual feeder samples, a subsample (~33 g) from each individual feeder was pooled within dietary treatment and sampling technique to form a single composite sample (~200 g). This process was repeated until 4 individual composite samples were created for each diet and sampling technique. Results reported herein, indicated that the observed variability when sampling feeders with an open handle probe was reduced (P = 0.013) for Cu and marginally reduced (P = 0.058) for Ca when compared with hand-sampling. However, no evidence for differences was detected among sampling techniques for Zn and P for the individual feeder analysis. There was no evidence for differences detected among sampling techniques for Cu, Zn, Ca, and P when samples were pooled from 6 feeders to form a single composite sample. From these results, sampling frequency calculations were determined to assess sampling accuracy

within a 95% confidence interval. Results indicated that the number of feeders or composite samples required to analyze was less regardless of Cu, Zn, Ca, and P when using a probe compared to a hand. In summary, these results would suggest that in general, sampling with a probe is associated with less variability on an individual sample basis, but when individual samples are pooled to form a composite sample, there was no difference among sampling techniques. Our results would suggest that samples collected with a probe and composited would be the best option to minimize variation and analytical costs.

Key words: calcium, copper, diet sampling, phosphorous, zinc

INTRODUCTION

The implementation and monitoring of quality control and quality assurance systems and their standard operating procedures in feed mill operations are integral in assessing the overall success and profitability of livestock operations (Richardson, 1996). The proper sampling of finished feed and its subsequent analysis is a common standard operating procedure that is used for most swine nutrition studies to ensure that adequate diet manufacturing and delivery has been met. Thus, serving as a control measure for both nutritionists and feed mill managers.

Interestingly, while numerous research articles and bulletins have been published on how to collect a representative sample as well as others describing analytical or laboratory to laboratory variation, we are unaware of any studies to examine exactly how many samples to collect from the feeders or if they should be pooled or not to minimize analytical variation. Therefore, this study was designed to evaluate different sampling procedures and number of samples to collect from feeders within a swine facility to achieve an accurate assessment of nutrient fortification in swine diets.

MATERIALS AND METHODS

General

For this study, feed was manufactured at a commercial feed mill in southwestern Minnesota. Ingredients were added to a ribbon mixer (Scott Model 6013, New Prague, MN) in 2,722 kg batches and mixed for 60 sec. These mash diets were then transported and delivered to a commercial grow-finish swine barn. The barn contained 42 pens that were each equipped with 1 cup waterer and a 4-hole stainless-steel, dry self-feeder (0.97 m tall and 1.52 m long; Thorp Equipment, Thorp, WI) with approximately 130 kg of feed capacity. Feed additions to each individual pen were made and recorded by a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN).

Experimental Design and Diets

A total of 36 feeders were used with 6 feeders per dietary treatment. This study was carried out as a split-plot design with the whole plot using 1 of 6 dietary Cu concentrations ranging from 27 to 147 mg/kg total Cu included in the diet, and the subplot using 1 of 2 sampling techniques from each feeder (probe vs. hand grab). The 6 dietary treatments (Table 5-1) consisted of: 3 corn-soybean meal-based diets with 20% corn DDGS formulated to contain 0.91% SID Lys, and 33, 87, or 147 mg/kg of total Cu or a second set of corn-soybean meal-based diets with 10% corn DDGS and formulated to contain 0.65% SID Lys and 27, 81, or 141 mg/kg of total Cu, respectively. Copper Sulfate (Prince Agri Products Inc., Quincy, IL) was added at 17, 70, and 130 mg/kg in diets A and D, B and E, and C and F, respectively. The remaining Cu making up the total Cu concentrations was provided by the corn, soybean meal, and corn DDGS. Nutrient profiles of the ingredients used in this study were based on NRC (2012) values.

Sampling and Chemical Analysis

Two sampling techniques (hand vs. probe) were tested on a total of 6 feeders per dietary treatment. The first sampling technique utilized was randomized within feeder. A 1.6 m brass open handle probe (Seedburo Equipment Company, Des Plaines, IL), which contained 10 openings spaced approximately 5.1 cm apart was utilized for this study. The probe was inserted at a 45° angle in relation to the bottom of the feeder, with slots facing upward and closed. After the probe was fully inserted, the slots were opened and the probe was moved up-and-down (~ 15.2 cm) in several short motions. The slots were then closed and the probe was removed from the feeder. Each sample obtained with a probe was approximately 250 g. Samples taken by hand were collected by inserting one's arm into the feeder at a depth of ~28 cm. Next, the individuals hand, wrist, and forearm were rotated so that their palm was facing upward toward the top of the feeder with their fingers placed together and slightly bent. The individual then lifted their arm out of the feeder. Each sample collected by hand was approximately 125 g. Each sampling technique was repeated within a feeder until approximately 900 g of sample was collected; approximately 4 times with the probe and 8 times by hand. To prevent cross contamination, the probe and individuals arm were wiped clean between feeders with a towel (Scott Shop Towel, Kimberly-Clark Worldwide, Inc., Dallas, TX). All samples were collected by the same individual. Samples were then transported back to the Kansas State University Swine Nutrition Lab (Manhattan, KS) where they were stored at -20ºC.

Samples were split using a riffle splitter (Humboldt Mfg. Co., Norridge, IL) and ground using a 0.5 mm screen (Retsch Ultra Centrifugal Mill ZM 200; Haan, Germany) prior to compositing and analysis. A 200 g subsample from each individual feeder and sampling technique was collected for analysis. In addition, a subsample (~33 g) from each individual

feeder and sampling technique was collected and pooled within dietary treatment and sampling technique to form a 200 g composite sample. This process was repeated until 4 individual composite samples were created for each diet and sampling technique. All samples were submitted to Cumberland Valley Analytical Services (Hagerstown, MD) for Cu, Zn, Ca, and P analysis (method 985.01; AOAC International, 2000) using a Perkin Elmer 5300 DV ICP (Perkin Elmer, Shelton, CT).

Statistical Analysis

Data were analyzed as a split-plot design, where the levels of the whole-plot treatment factor (diet) were assigned to feeders (i.e., the whole-plot experimental units) in a completely randomized design. The subplot treatment factor was sampling technique, and the feed sample collected via a given sampling technique was considered the subplot experimental unit. The duplicate assays on each feed sample were assumed to be subsamples. The concentrations of each analyte were fit to a linear mixed model using the PROC MIXED procedure in SAS (SAS Institute, Inc., Cary, NC) with the default estimation method REML. The Kenward-Roger method was used to adjust the denominator degrees of freedom and correct the standard errors for bias (Littell, et al., 2006). Diet, sampling technique, and diet × sampling technique interaction were modeled as fixed effects. Feeder nested within diet and the feeder × sampling technique interaction nested within diet were modeled as random effects. Mean concentrations of the various treatment combinations were computed using the LSMEANS statement.

A graphical analysis of the estimated random effects associated with the sampling techniques (results not shown here) indicated possible heterogeneous variances for the two sampling techniques. This need was formally assessed via a likelihood ratio test conducted by comparing the difference of the -2log likelihood statistics of the reduced model (with common

variance for sampling technique) and the full model (with a separate variance for each sampling technique) to a chi square distribution with 1 degree of freedom (Stroup, 2013). Heterogeneity was considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

Next, variance estimates from the appropriate model were used to calculate the number of samples needed to determine sampling accuracy with a given margin of error using a 95% confidence interval. To assess this, a margin of analysis was utilized where σ^2 = feeder variance + sampling technique variance + assay variance/2. It's important to note, that the variance for the residual from the covariance estimate was divided by 2 since each sample was analyzed in duplicate at the lab. We then calculated the margin of error from ± 2 ppm to ± 30 ppm using the observed variances for the hand and probe samples for the individual and composite feeder analysis.

RESULTS AND DISCUSSION

Results from mean chemical analysis of experimental diets can be found in Table 5-2. To determine whether the magnitude of differences between sampling techniques were significant, we used a chi-square analyses to evaluate the likelihood ratios comparing models accounting for heterogenous variance vs those that assumed homogenous variance. The observed variability (Table 5-3) when sampling feeders with an open handle probe was significantly reduced (P =0.013) for Cu (Figure 5-1) and marginally reduced (P = 0.058) for Ca (Figure 5-5) on the individual feeder analysis. There was no evidence for differences detected between sampling technique for Zn (Figures 5-3) and P (Figure 5-5) for the individual feeder analysis. Interestingly, when samples were pooled within sampling technique and dietary treatment to form a composite there was no evidence for differences detected between sampling techniques for Cu, Zn, Ca, and P. Thus, these results would suggest that pooling samples to form a homogenized composite

sample reduced total variability. Intuitively, this would be expected due to a homogenized sample being theoretically equal in composition throughout.

From these results, sampling frequency calculations were determined to assess sampling accuracy within a 95% confidence interval. To facilitate this, a margin of error analysis was utilized such that we wanted to estimate the mean concentration of a given diet with n samples and a margin of error (±) from the expected mean. Covariance parameter estimates generated from the heterogenous variances (full model) for Cu, Zn, Ca, and P were utilized in the calculation. Examples using the sampling frequency calculations are reported in Tables 5-4 and 5-5. For instance, if we wanted to estimate the mean concentration of 100 mg/kg Cu with a margin of error no larger or smaller than 15 mg/kg of Cu using a 95% confidence interval we would need to sample 17 feeders by hand and 7 feeders by probe when analyzing Cu on an individual feeder analysis. Based on our pooling of samples from 6 feeders we would need to submit 4 composite samples if sampling by hand and 2 composite samples if collected with a probe. Based on these results, it's clear that feed samples collected with probe require fewer feeders to be sampled. These results are in agreement with Reese and Miller (2006), who indicated that sampling feed using a grain probe was the most accurate sampling technique due to its ability to deeply penetrate into feeders, bags, and other containers obtaining samples from different locations. Thus, potentially accounting for potential feed particle segregation within the hopper as finer and dense particles tend to push-away lighter particles and settle down toward the bottom; whereas, larger and less dense particles rise to the top (Tang et al. 2006). Based on our results, a probed and pooled sample would lead to a lower number of samples and thus lower analytic cost for a given margin of error. One caution with the composite analysis is that this

applies to composites of 6 feeders. Further investigation is needed to determine the optimum number of feeders that would be needed to make the composite pools.

In conclusion, equations can be used to generate the sample size needed to accurately determine nutrient concentrations in a diet. Our results suggest that the best option to minimize variation and reduce analytical cost is to collect samples with a probe from 6 feeders and composite before analysis.

LITERATURE CITED

AOAC, 2000. Official Methods of Analysis of AOAC Int. 17th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS® for mixed models, 2nd ed. SAS Inst. Inc., Cary, NC.

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Reese, D. E., and P. S. Miller. 2006. Nutrient deficiencies and excesses. In: B. E. Straw, J. J. Zimmerman, S. D. Allaire, D. J. Taylor, editors. Diseases of swine. 9th ed. Blacknell Publishing, Oxford, UK. p. 931 – 944.

Richardson, C. R. 1996. Quality control in feed production. Proc. Mid-South Nutr. Conf. p. 1-5.

Stroup, W. W. 2013. Inference, part II: covariance components. In: F. Dominici, J. J. Faraway, M. Tanner, and J. Zidek, editors. Generalized Linear Mixed Models. CRC Press Taylor & Francis Group, Boca Raton, FL. p. 179 – 199.

Tang, P., P. H. Patterson, V. M. Purl. 2006. Effect of feed segregation on the commercial hen and egg quality. J. Appl. Poult. Res. 15:564-573. doi: 10.1093/japr/15.4.564.

Table 5-1. Diet composition, (as-fed basis)

	Diets[1]	
Item, %	A, B, and C	D, E, and F
Corn	61.33	79.48
Soybean meal, 46.0% CP	16.52	8.39
Corn DDGS[2]	20.00	10.00
Calcium carbonate	1.20	1.13
Monocalcium P, 21.5% P	---	0.09
Salt	0.35	0.35
L-Lys HCl	0.37	0.32
L-Thr	0.04	0.07
L-Trp	0.01	0.02
Phytase[3]	0.01	0.01
Trace mineral premix[4]	0.10	0.10
Vitamin premix[5]	0.08	0.05
Copper sulfate[6]	---	---
TOTAL	100	100
Calculated analysis		
Standardized ileal digestible (SID) amino acids, %		
Lys	0.91	0.65
Ile:Lys	62	59
Leu:Lys	159	166
Met:Lys	29	30
Met & Cys:Lys	56	59
Thr:Lys	61	65
Trp:Lys	18.5	18.5
Val:Lys	70	70
ME, kcal/lb	1,508	1,511
NE, kcal/lb	1,119	1,038
CP, %	18.1	12.9
Ca, %	0.55	0.50
P, %	0.40	0.34
Available P, %	0.26	0.22

[1]Diets A, B, and C were formulated for pigs ranging from 50 to 75 kg, while diets D, E, and F were for pigs ranging from 100 to 130 kg.

[2]Corn distillers dried grains with solubles.

[3]Optiphos 2000 (Huvepharma, Sofia, Bulgaria) provided 626 phytase units (FTU/kg) of diet with a release of 0.11% available P.

[4]Provided per kg of premix: Zinc 11,000 mg, Iron 11,000 mg, Manganese 3,000 mg, Copper 1,700 mg, Iodine 330 mg, and Selenium 300 mg.

[5]Provided per kg of premix: Vitamin A 7,054,720 IU, Vitamin D3 1,102,300 IU, Vitamin E 35,274 IU, Vitamin B12 26 mg, Riboflavin (B2) 6,173 mg, Niacin 39,683 mg, d-Pantothenic acid 22,046 mg, Menidione 3,527 mg per kg.

[6]$CuSO_4$, Copper Sulfate (Prince Agri Products Inc., Quincy, IL) were added at 17, 70, and 130 ppm in diets A and D, B and E, and C and F, respectively.

Table 5-2. Chemical analysis of dietary treatments for Cu, Zn, Ca, and P[1,2]

	Dietary Treatment					
Item	A	B	C	D	E	F
Individual analysis[3]						
Cu, ppm	53 (33)	124 (87)	155 (147)	51 (27)	96 (81)	150 (141)
Zn, ppm	159 (127)	154 (127)	162 (127)	139 (121)	165 (121)	141 (121)
Ca, %	0.83 (0.55)	0.98 (0.55)	0.91 (0.55)	0.73 (0.50)	0.67 (0.50)	0.64 (0.50)
P, %	0.51 (0.40)	0.51 (0.40)	0.50 (0.40)	0.39 (0.40)	0.39 (0.40)	0.40 (0.40)
Composite analysis[4]						
Cu, ppm	55 (33)	110 (87)	163 (147)	66 (27)	88 (81)	151 (141)
Zn, ppm	151 (127)	154 (127)	145 (127)	153 (121)	145 (121)	139 (121)
Ca, %	0.78 (0.55)	0.83 (0.55)	0.92 (0.55)	0.58 (0.50)	0.63 (0.50)	0.56 (0.50)
P, %	0.51 (0.40)	0. 50 (0.40)	0.51 (0.40)	0.42 (0.40)	0.41 (0.40)	0.41 (0.40)

[1]All dietary samples were submitted to Cumberland Valley Analytical Services (Hagerstown, MD) for analysis. Values reported are the means for each mineral for both hand and probe samples.

[2]Values in parenthesis indicate formulated values.

[3]Complete diet samples were collected from 6 feeders and placed into a 1 gallon sampling bag that was labeled with pen number, diet, and sampling technique.

[4]A subsample from each individual feeder and sampling technique was collected and pooled with dietary treatment and sampling technique to form a composite sample. This process was repeated until 4 individual composite samples were created for each diet and sampling technique.

Table 5-3. Evaluation for difference in variance between hand and probe sampling technique on Cu, Zn, Ca, and P using a chi-square test based on the likelihood ratio[1]

Analysis[2]	Parameters[3]	Chi square statistic[4]	Probability, *P* <
Cu			
Individual Feeder	Hand vs. Probe	6.2	0.013
Composite Sample	Hand vs. Probe	0.3	0.584
Zn			
Individual Feeder	Hand vs. Probe	0.1	0.752
Composite Sample	Hand vs. Probe	0.0	1.000
Ca			
Individual Feeder	Hand vs. Probe	3.6	0.058
Composite Sample	Hand vs. Probe	1.1	0.294
P			
Individual Feeder	Hand vs. Probe	0.4	0.527
Composite Sample	Hand vs. Probe	1.5	0.221

[1]The likelihood ratio test for covariance parameter estimates is a statistical test used to compare the goodness of fit of the heterogenous variance model allowing us to partition out the variances attributed to each sampling technique (hand and probe) to the homeogenous variance model that assumes the variances are the same.

[2]Mineral analysis on an individual feeder basis refers to the chi-square test based on the likelihood ratio from 6 individual feeders per dietary treatment and sampling technique. Whereas, the analysis on a composite feeder basis refers to the chi-square test based on the likelihood ratio when a subsample from each individual feeder was pooled within dietary treatment and sampling technique to form a single composite sample with a total of 4 composite samples created.

[3]Hand vs. Probe: samples were collected by inserting one's hand into a feeder or using inserting an open handle brass probe into feeders.

[4]Chi square statistic was calculated by taking the difference between the restricted log likelihood of the heterogenous variance model and restricted log likelihood of homogenous variance model.

Table 5-4. Sample size calculations for a given margin of error and a 95% confidence interval[1]

	Cu				Zn			
	Individual feeder analysis[2]		Composite feeder analysis[3]		Individual feeder analysis[2]		Composite feeder analysis[3]	
Margin of	Hand[4]	Probe[5]	Hand[4]	Probe[5]	Hand[4]	Probe[5]	Hand[4]	Probe[5]
Error, mg/kg	No. of feeders[6]		No. of samples[7]		No. of feeders[6]		No. of samples[7]	
± 2	967	375	220	140	306	268	140	135
± 4	242	94	55	35	77	67	35	34
± 6	107	42	24	16	34	30	16	15
± 8	60	23	14	9	19	17	9	8
± 10	39	15	9	6	12	11	6	5
± 15	17	7	4	2	5	5	2	2
± 20	10	4	2	1	3	3	1	1
± 25	6	2	1	1	2	2	1	1
± 30	4	2	1	1	1	1	1	1

[1]Values are calculated on the covariance parameter estimates obtained from the likelihood ratio test from the sampling and analysis of 6 feeders per dietary treatment.

[2]Individual feeder analysis: samples analyzed on an individual feeder basis.

[3]Composite feeder analysis: samples analyzed on 4 composite samples.

[4]Hand: samples taken by inserting one's hand into a feeder.

[5]Probe: samples taken with a 1.6 m open handle brass probe into a feeder.

[6]No of feeders: refers to the number of feeders that would need to be sampled to be with (±) a given margin of error on an individual feeder analysis basis.

[7]No of samples: refers to the number of composite samples needed when pooling samples across 6 feeders to be within a given margin of error.

Table 5-5. Sample size calculations for a given margin of error and a 95% confidence interval[1]

	Ca				P			
	Individual feeder analysis[2]		Composite feeder analysis[3]		Individual feeder analysis[2]		Composite feeder analysis[3]	
Margin of Error, %	Hand[4]	Probe[5]	Hand[4]	Probe[5]	Hand[4]	Probe[5]	Hand[4]	Probe[5]
	No. of feeders[6]		No. of samples[7]		No. of feeders[6]		No. of samples[7]	
± 2	169	84	87	53	4	5	4	2
± 4	42	21	22	13	1	1	1	1
± 6	19	9	10	6	1	1	1	1
± 8	11	5	5	3	1	1	1	1
± 10	7	3	3	2	1	1	1	1
± 15	5	2	2	1	1	1	1	1
± 20	3	2	2	1	1	1	1	1
± 25	3	1	1	1	1	1	1	1
± 30	2	1	1	1	1	1	1	1

[1]Values are calculated on the covariance parameter estimates obtained from the likelihood ratio test from the sampling and analysis of 6 feeders per dietary treatment and the pooling of the 6 feeders to form 4 composite samples.

[2]Individual feeder analysis: samples analyzed on an individual feeder basis.

[3]Composite feeder analysis: samples analyzed on 4 composite samples.

[4]Hand: samples taken by inserting one's hand into a feeder.

[5]Probe: samples taken with a 1.6 m open handle brass probe into a feeder.

[6]No. of feeders: refers to the number of feeders that would need to be sampled to be with (±) a given margin of error on an individual feeder analysis basis.

[7]No. of samples: refers to the number of composite samples needed when pooling samples across 6 feeders to be within a given margin of error

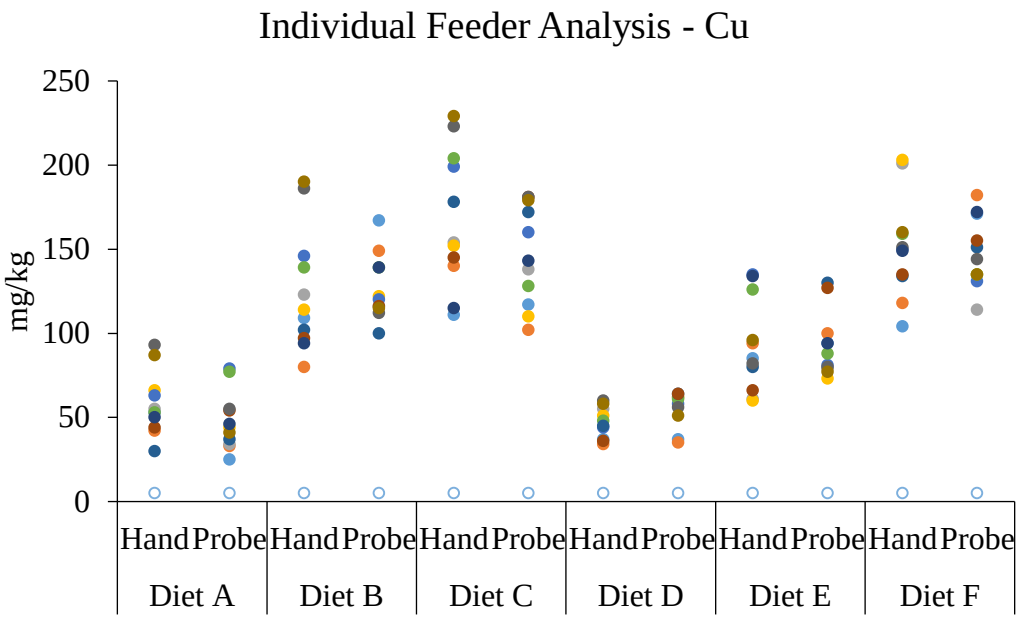

Figure 5-1. Distribution of analyzed mean Cu concentrations on an individual feeder basis. Each data point represents a single analysis of the 6 feeders in addition to its duplicate analysis for a total of 12 data observations for each sampling technique (hand: samples obtained by hand; probe: samples obtained using a 1.6 m open handle probe) within a given dietary treatment. Diets A, B, and C contained 33, 87, and 147 mg/kg of total Cu; whereas, diets D, E, and F contained 27, 81, and 141 mg/kg of total Cu.

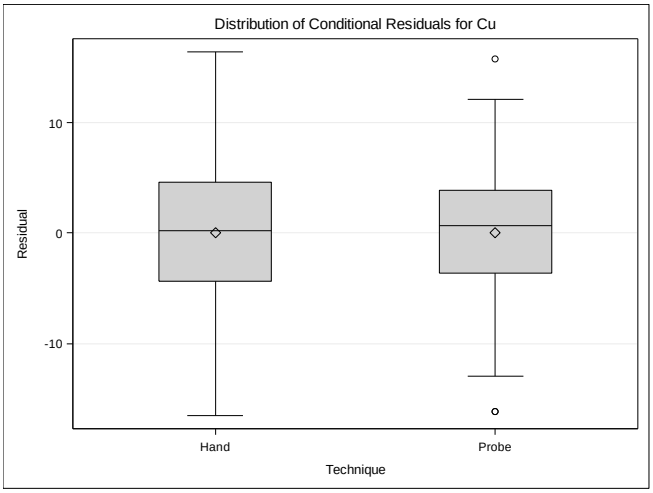

Figure 5-2. Statistical comparison examining the amount of variability attributed to samples obtained by hand and samples collected using a probe for Cu on an individual feeder basis.

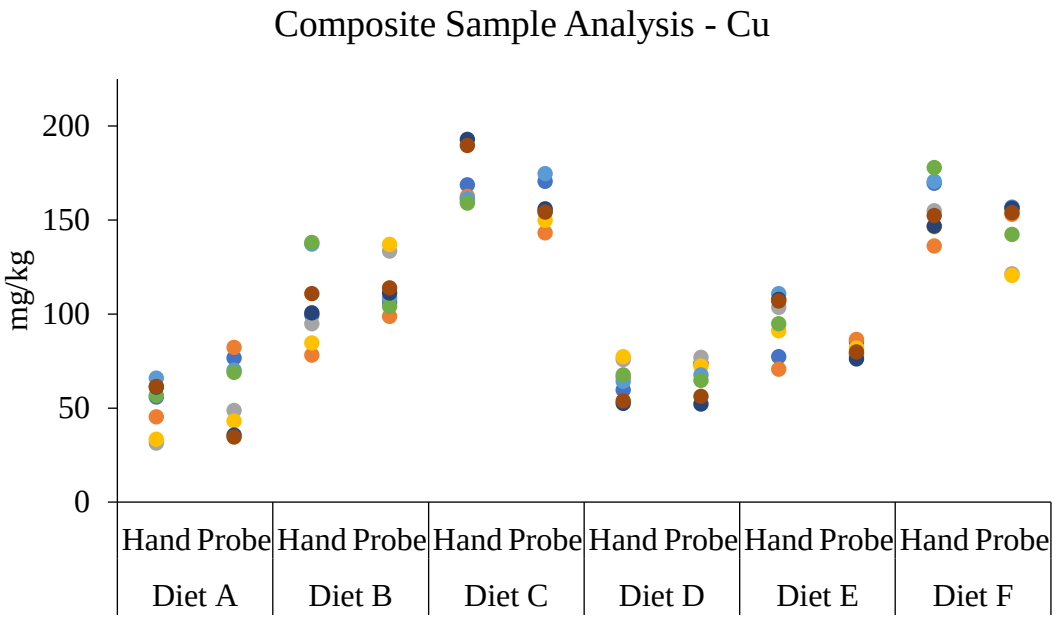

Figure 5-3. Distribution of analyzed mean Cu concentrations on a composite analysis basis in which a subsample from each of the 6 individual feeders were pooled within dietary treatment and sampling technique to form a single composite sample. The process was repeated until 4 individual composite samples were created for each diet and sampling technique. Each data point represents a single analysis on a composite sample in addition to its duplicate analysis for a total of 8 observations for each sampling technique (hand: samples obtained by hand; probe: samples obtained using a 1.6 m open handle probe) within a given dietary treatment. Diets A, B, and C contained 33, 87, and 147 mg/kg of total Cu, whereas, diets D, E, and F contained 27, 81, and 141 mg/kg of total Cu.

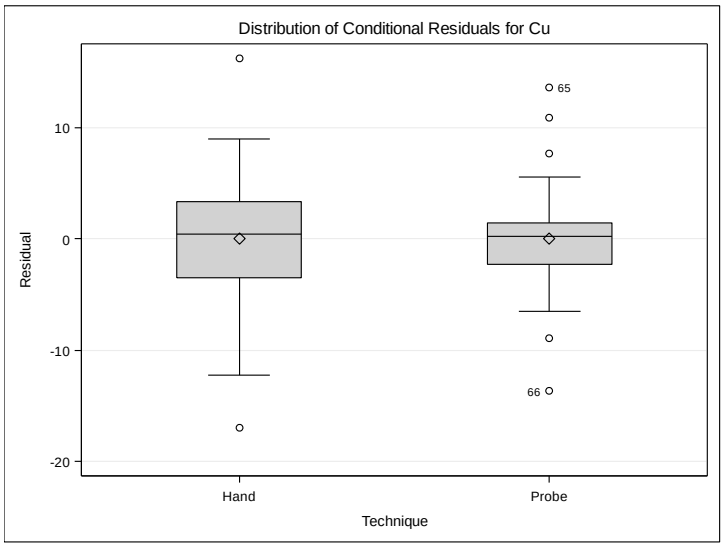

Figure 5-4. Statistical comparison examining the amount of variability attributed to samples obtained by hand and samples collected using a probe for the composite analysis of Cu.

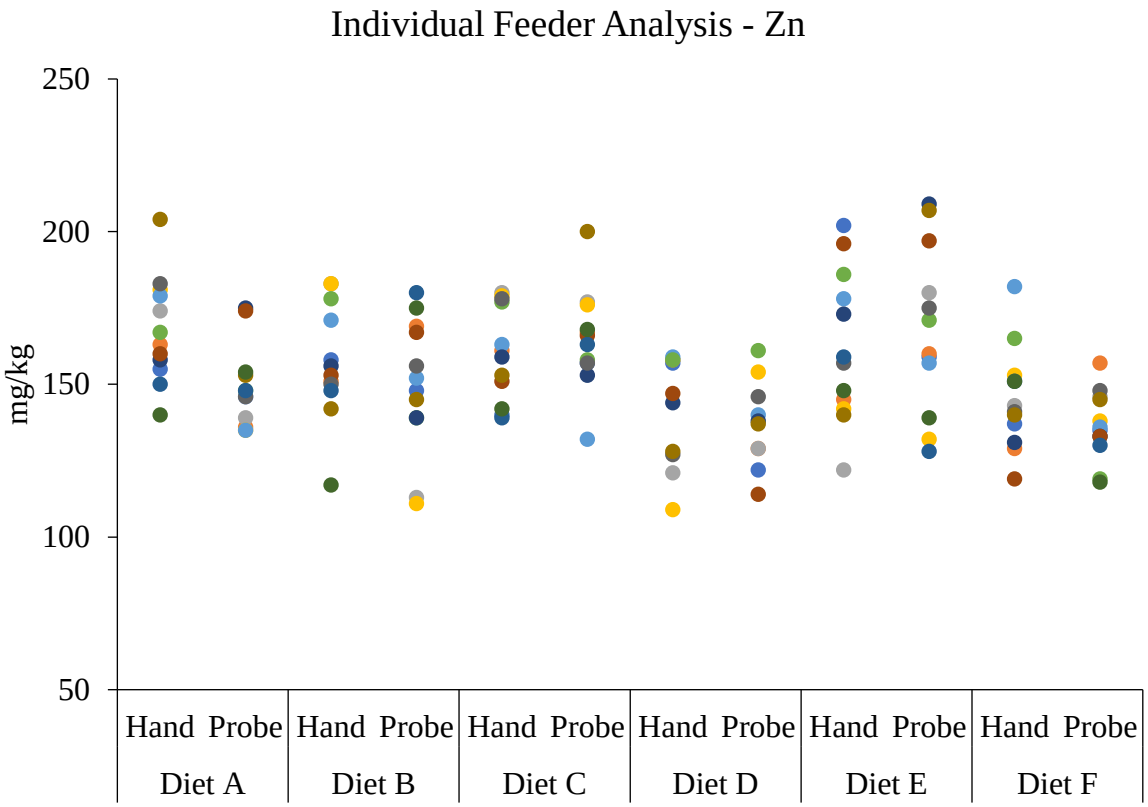

Figure 5-5. Distribution of analyzed mean Zn concentrations on an individual feeder basis. Each data point represents a single analysis of the 6 feeders in addition to its duplicate analysis for a total of 12 data observations for each sampling technique (hand: samples obtained by hand; probe: samples obtained using a 1.6 m open handle probe) within a given dietary treatment. Diets A, B, and C contained 127 mg/kg Zn; whereas, diets D, E, and F contained 121 mg/kg Zn.

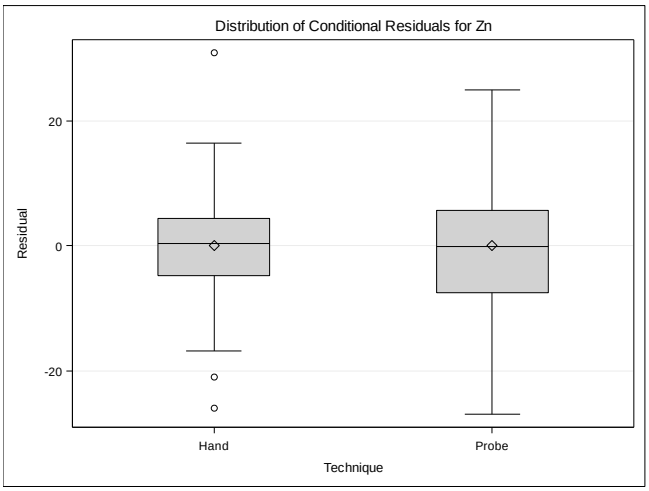

Figure 5-6. Statistical comparison examining the amount of variability attributed to samples obtained by hand and samples collected using a probe for Zn on an individual feeder basis.

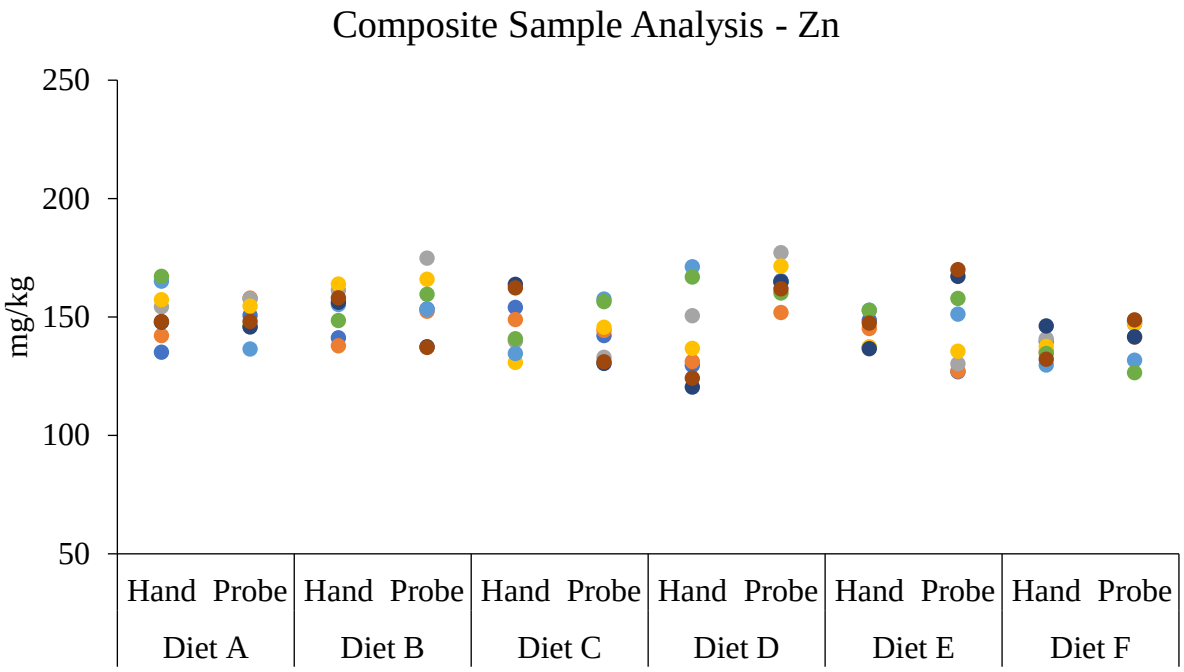

Figure 5-7. Distribution of analyzed mean Zn concentrations on a composite analysis basis in which a subsample from each of the 6 individual feeders were pooled within dietary treatment and sampling technique to form a single composite sample. The process was repeated until 4 individual composite samples were created for each diet and sampling technique. Each data point represents a single analysis on a composite sample in addition to its duplicate analysis for a total of 8 observations for each sampling technique (hand: samples obtained by hand; probe: samples obtained using a 1.6 m open handle probe) within a given dietary treatment. Diets A, B, and C contained 127 mg/kg Zn, whereas, diets D, E, and F contained 121 mg/kg Zn.

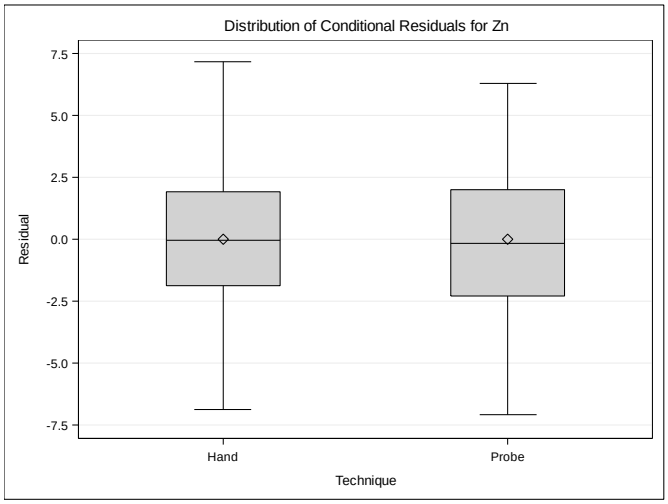

Figure 5-8. Statistical comparison examining the amount of variability attributed to samples obtained by hand and samples collected using a probe for the composite analysis of Zn.

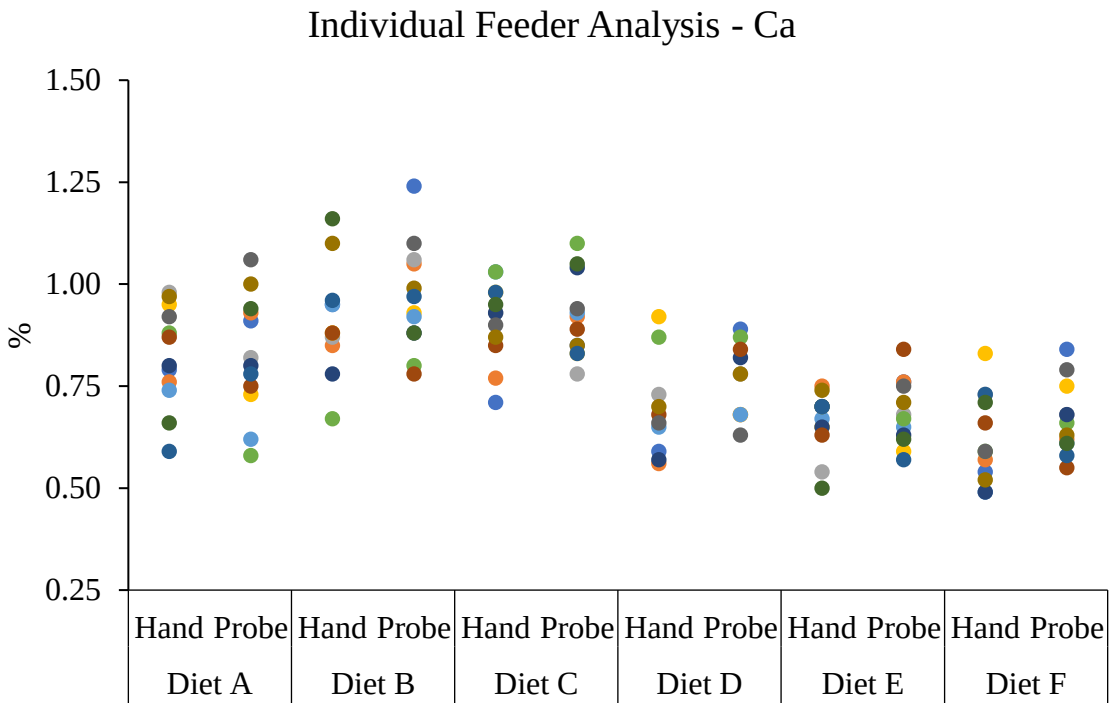

Figure 5-9. Distribution of analyzed mean Ca concentrations on an individual feeder basis. Each data point represents a single analysis of the 6 feeders in addition to its duplicate analysis for a total of 12 data observations for each sampling technique (hand: samples obtained by hand; probe: samples obtained using a 1.6 m open handle probe) within a given dietary treatment. Diets A, B, and C contained 0.55% Ca; whereas, diets D, E, and F contained 0.50% Ca.

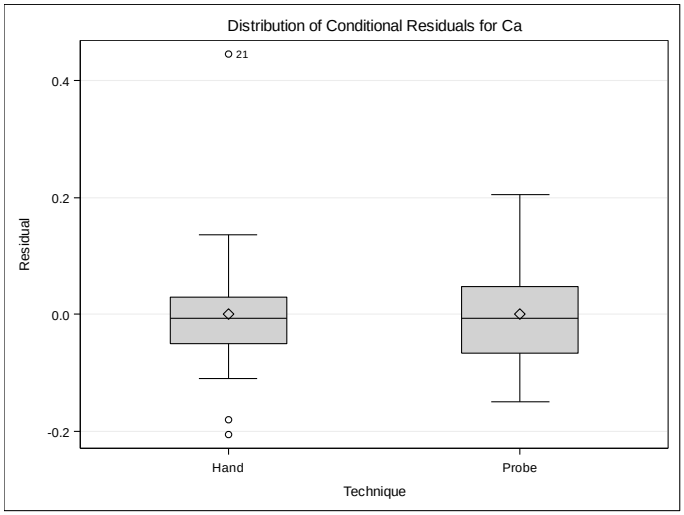

Figure 5-10. Statistical comparison examining the amount of variability attributed to samples obtained by hand and samples collected using a probe for Ca on an individual feeder basis.

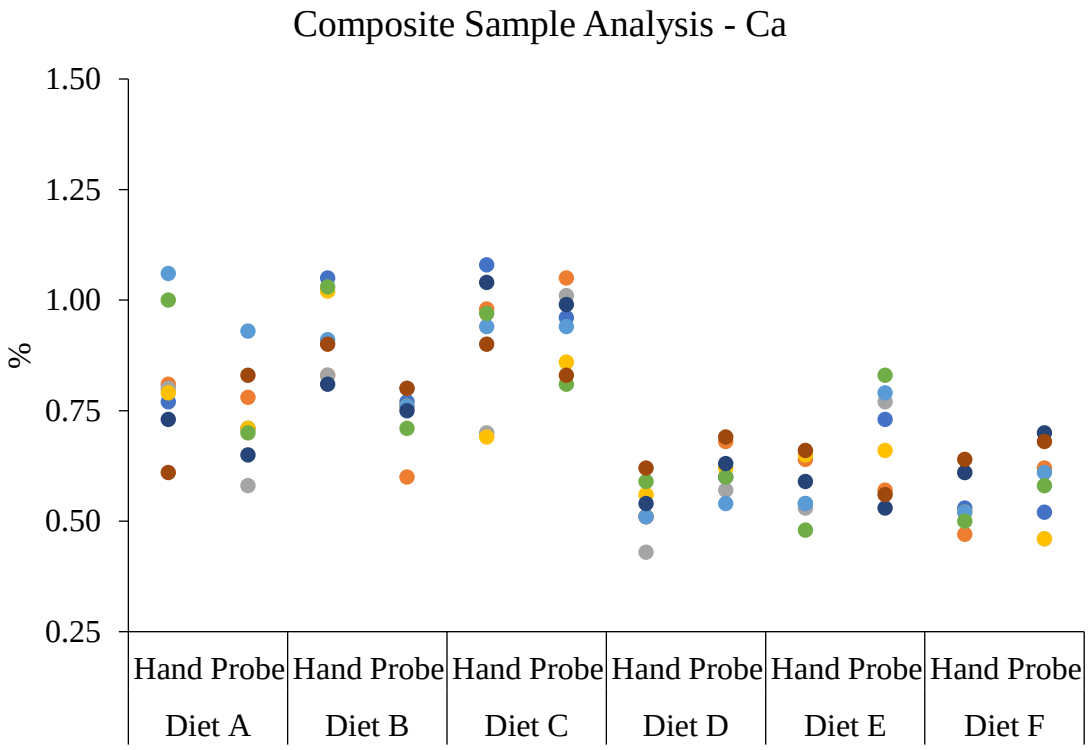

Figure 5-11. Distribution of analyzed mean Ca concentrations on a composite analysis basis in which a subsample from each of the 6 individual feeders were pooled within dietary treatment and sampling technique to form a single composite sample. The process was repeated until 4 individual composite samples were created for each diet and sampling technique. Each data point represents a single analysis on a composite sample in addition to its duplicate analysis for a total of 8 observations for each sampling technique (hand: samples obtained by hand; probe: samples obtained using a 1.6 m open handle probe) within a given dietary treatment. Diets A, B, and C contained 0.55% Ca, whereas, diets D, E, and F contained 0.50% Ca.

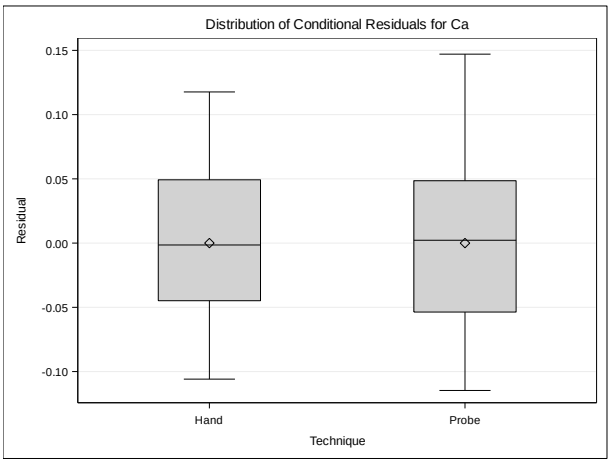

Figure 5-12. Statistical comparison examining the amount of variability attributed to samples obtained by hand and samples collected using a probe for the composite analysis of Ca.

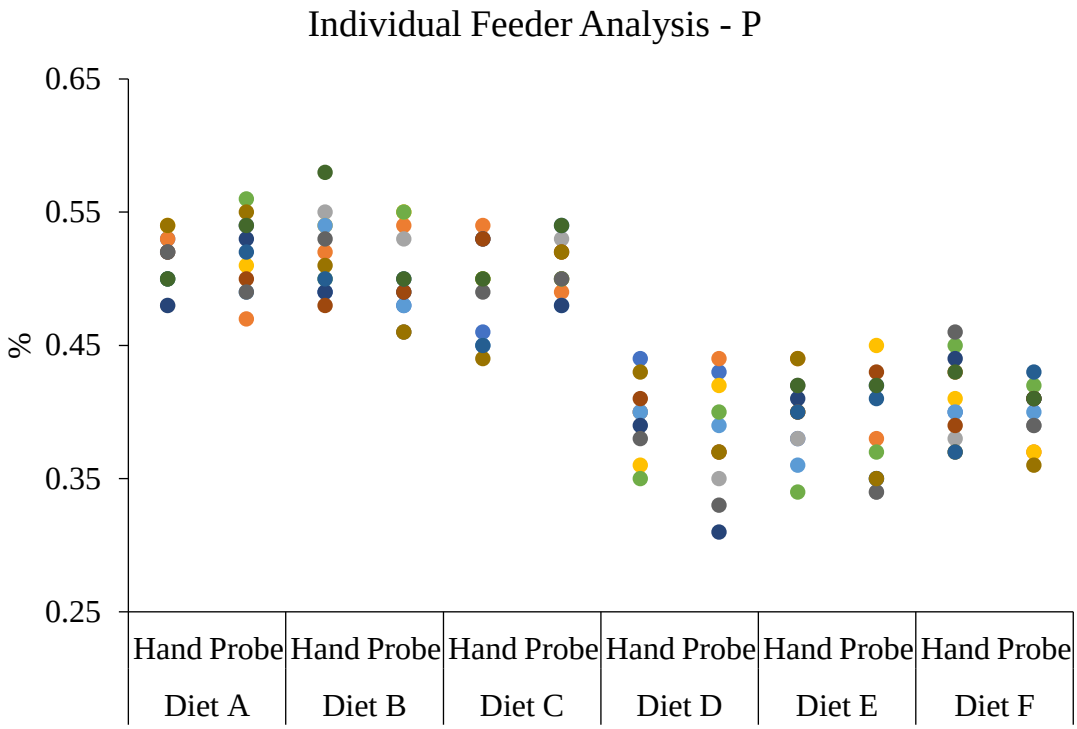

Figure 5-13. Distribution of analyzed mean P concentrations on an individual feeder basis. Each data point represents a single analysis of the 6 feeders in addition to its duplicate analysis for a total of 12 data observations for each sampling technique (hand: samples obtained by hand; probe: samples obtained using a 1.6 m open handle probe) within a given dietary treatment. Diets A, B, C, D, E, and F contained 0.40% P.

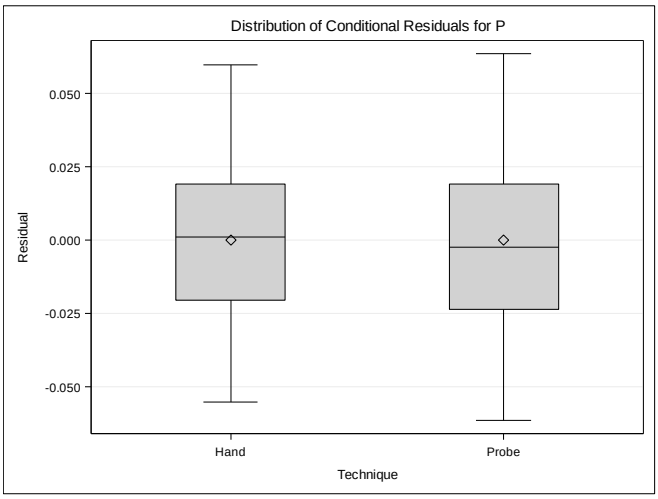

Figure 5-14. Statistical comparison examining the amount of variability attributed to samples obtained by hand and samples collected using a probe for P on an individual feeder basis.

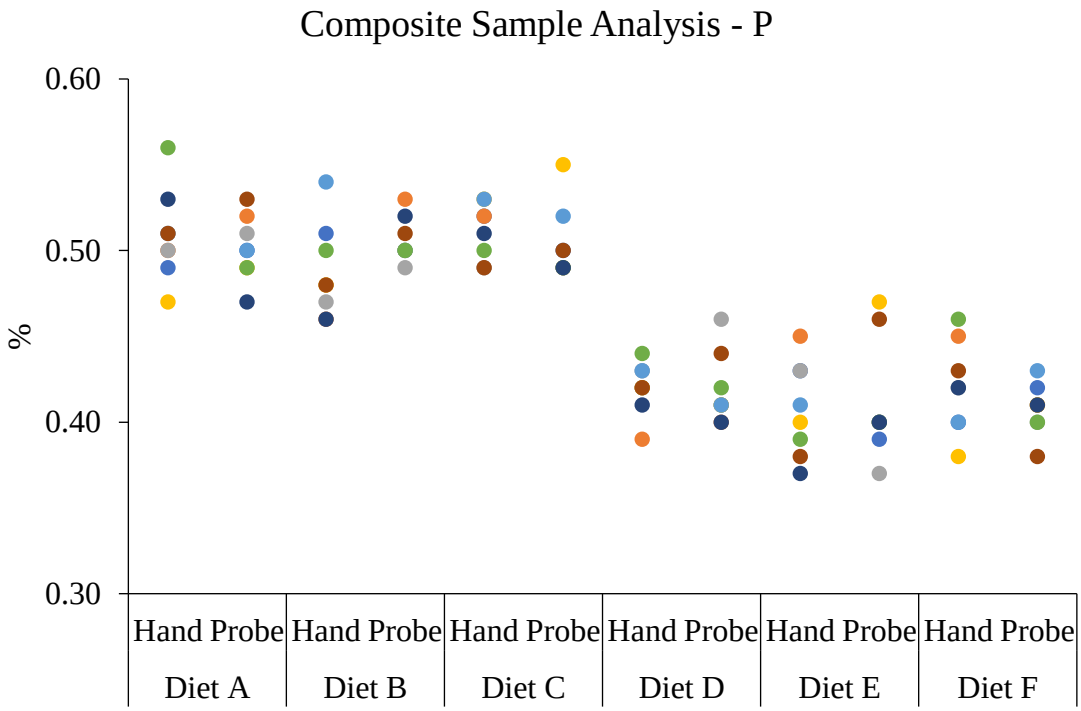

Figure 5-15. Distribution of analyzed mean P concentrations on a composite analysis basis in which a subsample from each of the 6 individual feeders were pooled within dietary treatment and sampling technique to form a single composite sample. The process was repeated until 4 individual composite samples were created for each diet and sampling technique. Each data point represents a single analysis on a composite sample in addition to its duplicate analysis for a total of 8 observations for each sampling technique (hand: samples obtained by hand; probe: samples obtained using a 1.6 m open handle probe) within a given dietary treatment. Diets A, B, C, D, E, and F contained 0.40% P.

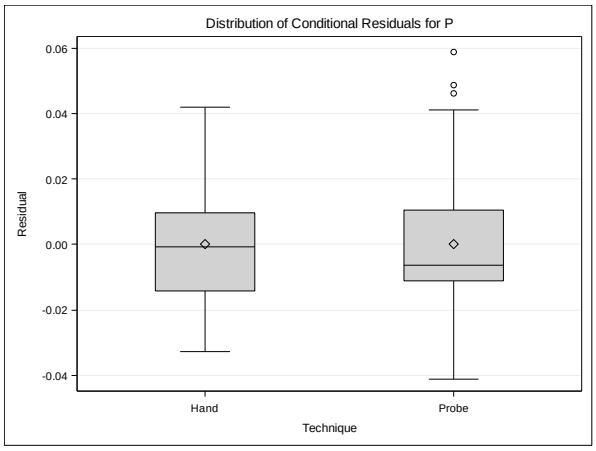

Figure 5-16. Statistical comparison examining the amount of variability attributed to samples obtained by hand and samples collected using a probe for the composite analysis of P.