Dietary acidifiers and nutritional value of soybean processing by-products for swine

by

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B.S., University of Illinois, 2019
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AN ABSTRACT OF A DISSERTATION

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DOCTOR OF PHILOSOPHY

Department of Animal Science and Industry
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Abstract

A total of 8 experiments structured in 6 chapters were performed to evaluate the impacts of in-feed acidifiers in nursery and grow-finish diets, develop a phytase release curve, and assess the composition and nutritional value of soybean processing by-products in swine diets. Chapter 1 utilized 320 pigs to evaluate the effects on growth performance and bone characteristics of 12- to 24-kg nursery pigs and develop an aP release curve for Smizyme TS G5 2,500 included from 500 to 2,500 FTU/kg. Overall, pigs fed increasing phytase had increased average daily gain (ADG), average daily feed intake (ADFI), gain-to-feed ratio (G:F), and final weight. Additionally, pigs fed increasing phytase had increased bone ash weight, percentage bone ash, bone density, and bone P. The available P release curve generated for Smizyme TS G5 2,500 for percentage bone ash using data generated from three bones is: aP release, % = (0.228 × FTU/kg) ÷ (998.065 + FTU/kg). Chapter 2 utilized 4,618 pigs across three studies to evaluate different benzoic acid feeding strategies in nursery and finishing pig diets. Overall, pigs fed benzoic acid in post-weaning diets had improved growth performance but if removed from the diet in late nursery, pigs had decreased performance relative to those maintained on benzoic acid throughout the study. In finishing, inclusion of benzoic acid had inconsistent results with one study finding improvements in G:F while the other found a reduction in G:F. Chapter 3 utilized 2,560 pigs across two studies to evaluate the effects of increasing sodium diformate in both nursery and finishing pig diets. Overall, increasing sodium diformate has the potential to improve feed efficiency in the early nursey period but did not affect performance in the late nursery. Furthermore, feeding increasing sodium diformate improved ADG and increased ADFI after d 60 (~82 kg) in the finishing period, but carcass traits were not influenced. Chapter 4 provided a comprehensive literature review on soybean processing by-products and their use in swine and

poultry diets. Chapter 5 encompassed an industry survey from 14 soybean plants across 8 production companies in the US to evaluate the composition and variation of soybean processing by-products. These data suggest soybean gums had a greater acid hydrolyzed fat content and decreased moisture and volatile matter percentage than soybean soapstocks. Notably, there was considerable variation in by-product composition between processing plants indicating differences in processing procedures or incoming soybean quality. Furthermore, when soybean by-products were added back to soybean meal, there was an increase in ether extract, but no effects on crude protein. Ultimately, soybean meal containing greater than approximately 1.6% ether extract on a dry matter basis likely contains soybean by-products. Chapter 6 utilized 350 pigs to evaluate the effects of soybean gums and soapstocks added back to soybean meal on nursery pig performance. Overall, adding soybean processing by-products such as soybean gums or soybean soapstocks had minimal effects on nursery pig growth performance. However, there is a potential for improved ADG and apparent total tract digestibility of dry matter when soybean meal containing added gums is included in the diet.

The information generated evaluating a phytase release curve and the use of in-feed acidifiers in swine diets can be used by producers to help optimize efficient diet formulation. Furthermore, the information generated on the composition of soybean processing by-products and their use in swine diets allows for a better understanding of soybean meal quality across US soybean processing plants.

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Chapter 1 - Determining the phosphorus release curve for Smizyme TS G5 2,500 phytase from 500 to 2,500 FTU/kg in nursery pig diets

ABSTRACT

A total of 320 pigs (Line 241 × 600, DNA, Columbus, NE; initially 11.9 ± 0.22 kg) were used in a 21-d growth study to determine the available P (aP) release curve for Smizyme TS G5 2,500 (Barentz, Woodbury, MN). At approximately 19 d of age, pigs were weaned, randomly allotted to pens, and fed common starter diets. Pigs were blocked by average pen body weight (BW) and randomly allotted to 1 of 8 dietary treatments on d 18 post-weaning, considered d 0 of the study. Dietary treatments were derived from a single basal diet and ingredients including phytase, monocalcium P, limestone, and sand were added to create the treatment diets. Treatments included 3 diets containing increasing inorganic P from monocalcium P (0.11, 0.20, and 0.28% aP), or 5 diets with increasing phytase (500, 1,000, 1,500, 2,000, or 2,500 FTU/kg) added to the diet containing 0.11% aP. All diets were corn-soybean meal-canola meal-based and were formulated to contain 1.24% SID Lys, 0.30% phytate P, and an analyzed Ca:P ratio of 1.10:1. Prior to the beginning of the study, all pigs were fed a diet containing 0.11% aP for a 2-d period (d 16 to 18 post-weaning). At the conclusion of the study, 1 pig, closest to the mean weight of each pen, was euthanized and the right fibula, rib, and metacarpal were collected to determine bone ash, density, and total bone P. Bones were weighed while suspended in a vessel of water and the weights used to calculate bone density (Archimedes' principle). For bone ash, bones were processed using the non-defatted method. For the overall experimental period, pigs fed increasing inorganic P had increased (quadratic, $P \leq 0.033$) average daily gain (ADG), average daily feed intake (ADFI), and final BW and a tendency for increased (quadratic, $P \leq 0.090$) gain:feed ratio (G;F). Pigs fed increasing phytase had increased (quadratic, $P \leq 0.004$)

ADG, G:F, and final BW and increased (linear, $P = 0.019$) ADFI. For fibula, rib, and metacarpal characteristics, pigs fed increasing aP from inorganic P had increased (linear, $P < 0.001$) bone ash weight, percentage bone ash, bone density, and bone P concentration. Additionally, pigs fed increasing phytase had increased (linear or quadratic, $P < 0.05$) bone ash weight, percentage bone ash, bone density, and bone P. The available P release curve generated for Smizyme TS G5 2,500 for percentage bone ash using data generated from all three bones is: aP = (0.228 × FTU/kg) ÷ (998.065 + FTU/kg).

INTRODUCTION

Typical corn-soybean meal diets contain phytate which acts as a major storage form of P in plant-based ingredients (Eeckhout and Paepe, 1994; Selle and Ravindran, 2008). However, this form of P is largely unavailable to swine due to limited endogenous production of the phytate-degrading enzyme, phytase. Therefore, it is common for most swine diets to contain an exogenous microbial phytase (Selle and Ravindran, 2008). This decreases the use of expensive inorganic forms of P, reduces antinutritional properties of phytate, and minimizes P excretion (Simons et al., 1990; Woyengo and Nyachoti, 2013).

There are multiple phytase sources currently available to swine producers and nutritionists. However, the efficacy of phytase varies based on multiple factors including phytase origin, activity in digestive tract, affinity to phytate, and resistance to degradation. Smizyme TS G5 2,500 (Barentz, Woodbury, MN) is produced through *Escherichia coli* fermentation and is classified as a bacterial derived 6-phytase. Two previous studies have demonstrated the efficacy of Smizyme TS G5 2,500 through improved growth performance and increased bone ash as phytase increased from 150 to 1,000 FTU/kg and from 250 to 1,500 FTU/kg (Wensley et al., 2020b). Both studies found improvements in growth performance and bone parameters up to the

highest inclusion level. Therefore, there is potential for continued benefits above the 1,500 FTU/kg inclusion level presented in previous literature.

Due to the sensitivity of bones to dietary P concentration, percentage bone ash has become a common indicator of phytase efficacy (Gourley et al., 2018; Wensley et al., 2020b; Becker et al., 2021). However, as further research develops, there is potential that specific bones and bone measurements may provide better estimates of total body characteristics (Lee et al., 2021). Therefore, using multiple bones such as the fibula, rib, and metacarpal may provide a more robust estimate of aP. Additionally, using multiple bone measurements in addition to bone ash percentage including bone density, bone P, and histology can provide further insight on bone mineralization and sensitivity to dietary P. The objective of this study was to evaluate the effects on growth performance and bone characteristics of 12- to 24-kg nursery pigs and to develop an aP release curve for Smizyme TS G5 2,500 included from 500 to 2,500 FTU/kg.

MATERIALS AND METHODS

The protocol used in this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee. Corn, soybean meal, canola meal, limestone, and monocalcium phosphate were analyzed for Ca and P at the Kansas State University Soils Lab using inductively coupled plasma spectroscopy-optical emission spectroscopy after wet ash sample preparation (Method 985.01 A, B, and D; Method 942.05; AOAC, 2006) to determine nutrient loading values for diet formulation (Table 1.1). Phytase for the experiment was analyzed (Eurofins Scientific Inc., Des Moines, IA) to determine inclusion rate and was found to contain 2,300,000 FTU/kg. There was no allowance for release of Ca by phytase and all diets were formulated to contain a Ca:P ratio of 1.10:1. Diets contained 7.5% canola meal, and because of its high phytate P concentration, resulted in a diet with a calculated 0.30% phytate P (NRC,

2012). Diets were formulated to 1.24% SID Lys with other amino acids set to meet or exceed NRC (2012) requirement estimates as a ratio relative to Lys.

Diet manufacturing

A single base diet was manufactured at Hubbard Feeds in Beloit, KS (Table 1.2). For each experimental diet, treatment specific ingredients including limestone, monocalcium P, sand, and Smizyme TS G5 2,500 phytase were mixed with a subset of the basal diet at the O.H. Kruse Feed Technology Innovation Center in Manhattan, KS to obtain the final experimental diets. During bagging, complete diet samples were collected from every fourth bag, pooled, ground to 200 µm, and stored at -20°C until submitted for analysis.

Animals and Housing

The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Pigs were provided *ab libitum* access to feed and water with each pen containing a 4-hole, dry self-feeder and nipple waterer. A total of 320 pigs (Line 241 × 600, DNA, Columbus, NE) were weaned at approximately 19 d of age. Following weaning, pigs were randomly allotted to pens and fed common starter diets. Pigs were blocked by body weight (BW) (initially 11.9 ± 0.22 kg) and randomly allotted to 1 of 8 dietary treatments on d 18 post-weaning, considered d 0 of the study. There were 5 pigs per pen (3 barrows and 2 gilts or 2 barrows and 3 gilts) and 8 pens per treatment. Treatments included 3 diets containing increasing (0.11, 0.20, and 0.28%) inorganic P from monocalcium P, or 5 diets with increasing (500, 1,000, 1,500, 2,000, or 2,500 FTU/kg) phytase added to the diet containing 0.11% aP. Prior to the beginning of the study, all pigs were fed a diet containing 0.11% aP for a 2-d period (d 16 to 18 post-weaning).

Throughout the 21-d experiment, pig and feeder weights were measured every 7 d to determine average daily gain (ADG), average daily feed intake (ADFI) and gain:feed ratio (G:F). At the conclusion of the study, 1 pig, closest to the mean weight of each pen, was euthanized via penetrating captive bolt. The right fibula, 10th rib, and metacarpal were collected, placed in individual plastic bags with identification, and stored at -20°C until bone analysis. For bone analyses, leftover extraneous soft tissue and cartilage caps were removed from each bone. For bone density, Archimedes' principle was used. Bones were submerged in ultra-purified water under vacuum for 4 h. Bones were then weighed while suspended in a vessel of water and the weights used to calculate bone density. For bone ash, bones were processed using the non-defatted method. Each fibula was dried at 105℃ for 7 d in a drying oven and subsequently ashed at 600℃ for 24 h in a muffle furnace. This method was used to determine total bone ash weight and percentage ash relative to dried bone weight (Wensley et al., 2020a). For bone P, a portion of the residual bone ash was placed in a tube with nitric acid and incubated at 60℃ for 6 h. The solution was then diluted using ultra-purified water and P quantity measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Additionally, the left 10th rib was collected and stored in formalin for histologic evaluation at Iowa State Veterinary Diagnostic Laboratory. Three diagnostic pathologists performed histologic evaluation of hematoxylin and eosin-stained sections of the left 10th rib without knowledge of treatments. Scores were given for microscopic fractures and for lesions due to failure of endochondral ossification. A grade for physis, infractions, and fibrosis was determined for each sample. Scores ranged from 0 to 3 for physis and infraction scores with 0 indicating no histologic findings and 3 meaning severe histologic findings. Scores of 0 or 1 were provided for fibrosis score with 0 indicating no evidence of fibrosis and 1 indicating fibrosis in the medullary of the bone.

Chemical Analysis

Three samples per dietary treatment were submitted for duplicate Ca and P (Method 985.01 A, B, and D; Method 942.05; AOAC, 2006) analysis at the KSU Soils Lab in Manhattan, KS. Furthermore, two samples of each experimental diet were submitted for phytase analysis (Barentz, Woodbury, MN) using the AOAC official method 2000.12.

Statistical Analysis

Data were analyzed as a randomized complete block design with pen as the experimental unit, treatment as a fixed effect, and weight block as a random effect. The base model was evaluated using the MIXED procedure of SAS OnDemand for Academics (SAS Institute, Inc., Cary, NC). Linear and quadratic contrasts were constructed within increasing inorganic P or phytase treatments. Histological data were analyzed as categorical outcomes. A multinomial response distribution with a generalized linear mixed model using a cumulative logit link was used. Data were summarized and reported as percentage of observations within each score category by treatment.

Marginal intake of aP per day was calculated for each pen fed the inorganic P diets. The equation is as follows: aP intake/day = [dietary aP% - 0.11% (aP in the basal diet)] × ADFI. This served as the predictor value to develop a standard curve for each response criterion. The equation for the standard curve was used to calculate aP release from each pen fed the different phytase dosages based on the observed value for each response criterion. Using the pen ADFI, this value was then converted to a marginal aP%.

A mixed model ANOVA with weight block as the random effect was used to evaluate aP release as a function of the calculated phytase dosage. Additionally, to evaluate the average aP release generated using data from all three bones, bone was added as a fixed effect and pen

served as a random effect in the model. Formulated phytase levels were used to calculate all release values.

A model was fit to pen release values using non-linear regression using the following functional form:

$$Available\ P\ release, \% = \frac{a \times FTU}{b + FTU}$$

The coefficient *a* is a horizontal asymptote indicating the maximum release of aP for each response, and coefficient *b* indicating the vertical asymptote. The model parameters were estimated using the nls function from the R Stats Package [Version 4.1.1 (2021-08-10); R Core Team, 2021] using the RStudio environment (Version 1.4.1717; RStudio Team, 2021) in order to develop aP release curves for percentage bone ash and bone density. Results were considered to be significant with *P*-values ≤ 0.05 and were considered marginally significant with *P*-values ≤ 0.10.

RESULTS

Analysis of final diets for Ca, P, and phytase activity were similar to expectations based on diet formulation (Table 1.3). Phytase activity of complete diets increased across the phytase treatments with analyzed phytase concentrations of 519, 1,046, 1,507, 2,036, and 2,499 FTU/kg.

From d 0 to 21, pigs had increased (quadratic, $P \leq 0.033$) ADG, ADFI, and final BW when fed increasing aP from inorganic P (Table 1.4). Additionally, pigs fed increasing inorganic P had increased (linear, $P < 0.001$) G:F. Similarly, pigs fed increasing phytase had increased (quadratic, $P \leq 0.004$) ADG, G:F, and final BW. Furthermore, ADFI increased in a linear fashion ($P = 0.019$) for pigs fed increasing phytase.

For fibula, rib, and metacarpal characteristics, pigs fed increasing aP from inorganic P resulted in a linear increase ($P < 0.001$) for all bone properties except bone P percent. Rib bone

density also exhibited a quadratic increase ($P = 0.002$) as inorganic P increased. Pigs fed increasing aP from inorganic P had a tendency for increased rib (linear, $P = 0.065$) bone P percent and had a quadratic response ($P = 0.045$) for fibula bone P percent. However, there were no differences ($P > 0.10$) in metacarpal bone P percent as pigs were fed increasing aP from inorganic P.

For fibula characteristics, pigs fed increasing phytase had increased bone ash weight (linear, $P < 0.001$), percentage bone ash (quadratic, $P = 0.022$), bone density (quadratic, $P = 0.032$), and bone P content (linear, $P < 0.001$). Additionally, for rib characteristics, pigs fed increasing phytase had increased bone ash weight (quadratic, $P = 0.038$), percentage bone ash (quadratic, $P = 0.005$), bone density (quadratic, $P = 0.027$), and bone P content (linear, $P < 0.001$). For metacarpal characteristics, pigs fed increasing phytase had increased bone ash weight (linear, $P < 0.001$), bone ash percentage (quadratic, $P = 0.016$), bone density (quadratic, $P = 0.086$), and bone P (linear, $P < 0.001$). Inversely, there were no differences ($P > 0.10$) in bone P percent for the fibula, rib, or metacarpal as pigs were fed increasing phytase in the diet. For histopathology, there was no evidence of differences ($P > 0.10$) in physeal, infraction, or fibrosis score (Figures 1.1).

Based on these bone criteria, the calculated percentage aP released from Smizyme TS G5 2,500 varied depending on the growth performance, different bone, and bone criteria measured (Table 1.5). Overall, the calculated aP release values increased (quadratic, $P \leq 0.07$) for all response criteria. The calculated aP release values were then used to generate separate aP release curves for the fibula, rib, and metacarpal (Figure 1.2). Furthermore, additional aP release curves for percentage bone ash and bone density were generated using the average of the data from all three bones. A model was fit to determine the release values using non-linear regression

(Wensley et al., 2020b; Becker et al., 2021).

The release equations generated from this experiment accounting for the average of the fibula, rib and metacarpal for Smizyme TS G5 2,500 are (Figure 1.2):

$$Percentage\ bone\ ash\text{:}\ \ aP, \% = \frac{0.228 \times FTU/kg}{998.065 + FTU/kg}$$

$$Bone\ density\text{:}\ \ aP, \% = \frac{0.236 \times FTU/kg}{1{,}086.92 + FTU/kg}$$

DISCUSSION

Adding phytase to swine diets has become a common practice in swine nutrition over the past decade and has repeatedly shown to improve production, economics, and sustainability of the swine industry (Selle and Ravindran, 2008). Furthermore, optimizing P concentrations is important during the nursery phase in order to maintain animal growth and welfare. If adequate digestible P is not included in nursery diets, there is a potential for restricted growth, lameness, and decreased bone P reserves for later growing and finishing phases (Buhler et al., 2010; She et al., 2017; Wensley et al., 2020b). Due to the importance of adequate P content in swine diets and the varying abilities of phytase to degrade phytate, it is important to investigate the efficacy of new phytase sources. This efficacy is often evaluated using aP release curves (Gourley et al., 2018; Wensley et al., 2020b; Becker et al., 2021). This is due to STTD P representing the amount of phosphorus that is digested and absorbed while aP represents the amount of P that is digested, absorbed, and available for utilization in bone mineralization. Therefore, by determining the aP of a phytase, the industry can further understand the cumulative effect of a specific phytase when fed to pigs (Zhai et al., 2023).

Phytase was originally derived from the fungi *Aspergillus ficuum* and included in swine diets for the first time in 1990 (Simons et al., 1990). As phytase became commonly used in swine

diets, there was an increased effort to develop new phytase sources with improved functionality. Phytase derived from the bacteria *Escherichia coli* was developed to have a greater affinity for phytate and is now one of the most used phytases in monogastric diets (Selle and Ravindran, 2008). *Escherichia coli* derived phytase is part of the histidine acid phosphatase family. These phytases exhibit a stronger specificity for phytate than other phytase sources on the market (Wodzinski and Ullah, 1996; Lim et al., 2000). Wyss et al. (1999) reported that the activity of *Escherichia coli* derived phytase was approximately 800% greater than the activity of phytase derived from *Aspergillus niger* (Wyss et al., 1999; Lim et al., 2000). As the development of phytase continues, there is a specific focus on increased resistance to proteolytic enzymes and thermal tolerance (Selle and Ravindran, 2008). The phytase used in this experiment, Smizyme TS G5, is an *Escherichia coli* derived 6-phytase that is expressed in Pichia Pastoris yeast. This generation of phytase was developed with the goal of exhibiting improved thermal stability and pH specificity.

Wensley et al. (2020b) conducted two experiments to determine the P release of Smizyme TS G5 2,500 phytase. In their first experiment investigating levels of phytase from 150 to 1,000 FTU/kg, they found increased growth performance and bone criteria up to the highest phytase inclusion. Additionally, a second experiment investigated levels of phytase from 250 to 1,500 FTU/kg. Like the first experiment, there was an increase in all growth performance and bone criteria up to the highest inclusion level. In alignment with previous studies, the current study saw an increase in all growth performance measures and bone parameters in either a linear or quadratic manner depending on response. However, unlike the previous studies, the maximum response for most parameters measured was not at the highest inclusion level. In the current study, ADFI and final BW were maximized at 500 and 2,000 FTU/kg, respectively. Percentage

bone ash and bone density were maximized between 1,500 and 2,000 FTU/kg. Therefore, the potential maximum benefit when using Smizyme TS G5 2,500 may be seen at inclusion levels below 2,500 FTU/kg. Furthermore, previous, and current research agree that the estimated aP released by Smizyme TS G5 2,500 varied depending on the selected response criteria due to their sensitivity to changes in aP within the body. Overall, the calculated aP release values for bone ash percentage generated using data from all three bones from the current study are lower at both the 500 and 1,000 FTU/kg inclusion levels, but similar at the 1,500 FTU/kg inclusion level when compared to the previous studies.

Unlike previous studies investigating the effects of Smizyme TS G5 2,500 in swine diets, the current study utilized canola meal in the diets to provide increased phytate to understand the aP release value of Smizyme at higher inclusion levels without limiting the response by the quantity of phytate available. Canola meal has been shown to have a high level of reactive phytate in studies conducted with broilers (David et al., 2021). Selle and Ravindran (2008) reported that canola meal has a total P concentration of 9.72 g/kg with 66.4% of the total P comprised as phytate P. Furthermore, canola meal has approximately 2.57 g/kg more phytate P than soybean meal commonly used in swine diets (Selle and Ravindran, 2008). Therefore, the diets in this study were formulated to contain approximately 0.30% phytate P whereas a typical corn-soybean meal diet would contain approximately 0.24% phytate P.

Phosphorus is the second most abundant mineral in the body, and it plays important roles in multiple physiological functions and bone mineralization (NRC, 2012). It has been well documented that the P needed to maximize lean growth is independent of the P needed to maximize bone strength and mineralization (Cromwell et al., 1970; Augspurger et al., 2003; Wensley et al., 2020b). The NRC (2012) determined that at least 0.1 percentage units more Ca

and P are needed to maximize bone strength compared to growth performance. Based on this knowledge, evaluating bone strength and mineralization has become a common practice in phytase studies (Gourley et al., 2018; Wensley et al., 2020b; Becker et al., 2021). However, as increased information on the use of various bones and analytical methods develops (Wensley et al., 2020a; Lee et al., 2021; Williams et al., 2022); the information can be used to help best guide procedures used when evaluating efficacy and aP release values for novel phytase sources. A recent study investigated the effects of different bones and analytical methods on assessment of bone mineralization in response to dietary P, phytase, and vitamin D in 12 kg pigs using the metacarpal, fibula, 2nd rib, and 10th rib (William et al., 2022). Response to the different treatments was dependent on type of bone with the 2nd rib and fibula showing the greatest sensitivity to P. In contrast, Crenshaw et al. (1981) observed that in young pigs (9 kg) the femur, humerus, and rib were the most sensitive to changes in P. However, they observed that as pigs aged, the sensitivity of the bones to P changed. On the final slaughter day of the study, the humerus, metacarpals, metatarsals, and vertebrae showed the greatest response to P levels in the diet (Crenshaw et al.,1981). Furthermore, a study by Lee et al. (2021) evaluating the correlation of various bones with total body bone ash in 40 kg pigs found that metacarpals, metatarsals, and tibias were most representative of total body P when compared to the femur, fibula, and ribs. Therefore, it is clear multiple factors can impact which bone is most sensitive to dietary P in swine diets. Unlike previous phytase release studies, the current study evaluated multiple bones (fibula, 10th rib, and metacarpal) using bone ash weight, bone ash percentage, bone density, and bone P measurements. As a result, an aP release equation was developed based on an average of the three bones for each response variable. Due to the differences in bone sensitivity to P, the aP

release values presented for the average of the three bones may provide a more robust estimate than the aP release data for individual bone parameters.

Both bone P weight in grams and bone P percent were collected to evaluate bone mineralization; however, they were not used to develop an aP release curve. The increase in bone P weight, but no changes in bone P percent as phytase increased in the diet aligns with past literature (Blavi et al., 2019; Lee et al., 2021). This is driven by the composition of bone P and Ca remaining relatively constant as dietary concentrations change; however, bone size is largely impacted. Therefore, the changes seen in bone P weight is largely driven by bone size rather than actual bone P composition. Therefore, bone ash percentage may still be the most accurate measure of aP release from phytase as it is independent of bone size.

In addition to the bone parameters collected to develop the aP release curves, the left 10^{th} rib was collected and used for histologic investigation. Although there were no statistical differences for any of the histologic measurements, there are important numerical differences. For all three measurements, the frequency of a score 0 increases numerically as more aP was included in the diet. The 0.28% aP diet had a frequency of 100% for a score 0 when evaluating both infraction and fibrosis grades indicating no signs of fractures or fibrosis. There is a similar numerical increase in the frequency of a score 0 as phytase inclusion increases. These numerical differences potentially indicate a healthier, better developing bone. This aligns with a study that evaluated tibial histology when utilizing two different forms of phytase (Zhang et al., 2000). They found increasing phytase in the diet produced a thinner bone physis indicating an improvement in bone development. Similarly, improvements in bone histology have been seen in broilers with birds fed phytase having a decreased hypertrophic zone width, but increased

proliferation zone width indicating improved bone development and mineralization compared to birds not fed phytase (Qian et al., 1996).

In summary, this study has provided aP release values for Smizyme TS G5 2,500 phytase in nursery pigs weighing 12- to 24-kg when fed at levels between 500 and 2,500 FTU/kg. The aP release at different FTU inclusion levels depended on response criterion. Equations for aP release were developed based on the different criterion measured. The aP release curve generated for Smizyme TS G5 2,500 for percentage bone ash using data generated from using the average of the fibula, rib, and metacarpal bones is: $aP = (0.228 \times FTU/kg) \div (998.065 + FTU/kg)$ and may provide the most robust estimate.

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Figure 1.1. Frequency of A) physeal grade B) infraction grade and C) fibrosis grade by treatment.

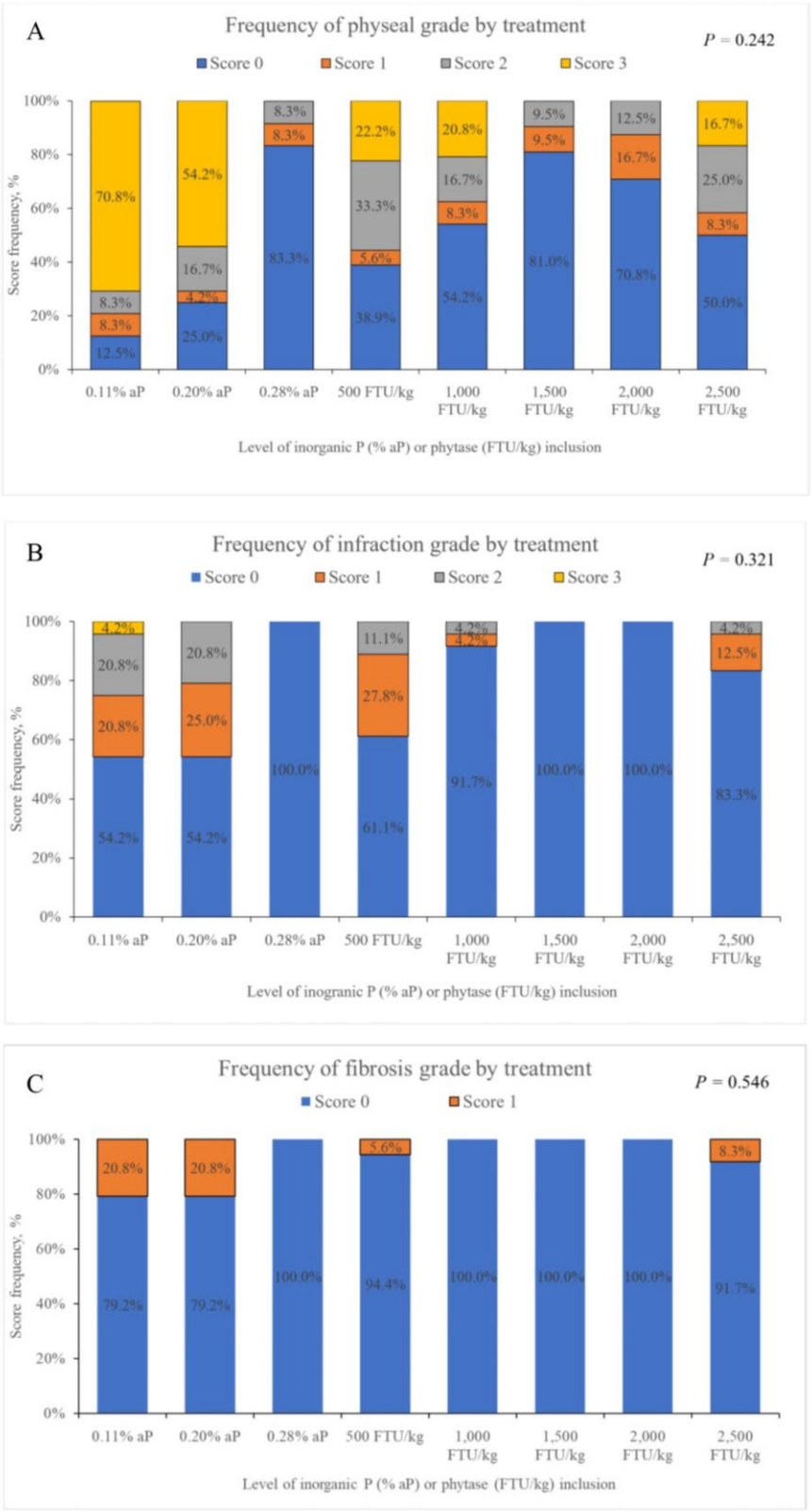

Figure 1.2 Available P release curves for A) right fibula B) right rib C) right metacarpal and D) average of all three bone characteristics including percentage bone ash and bone density generated using the release equations for Smizyme TS G5 2,500 from this experiment.

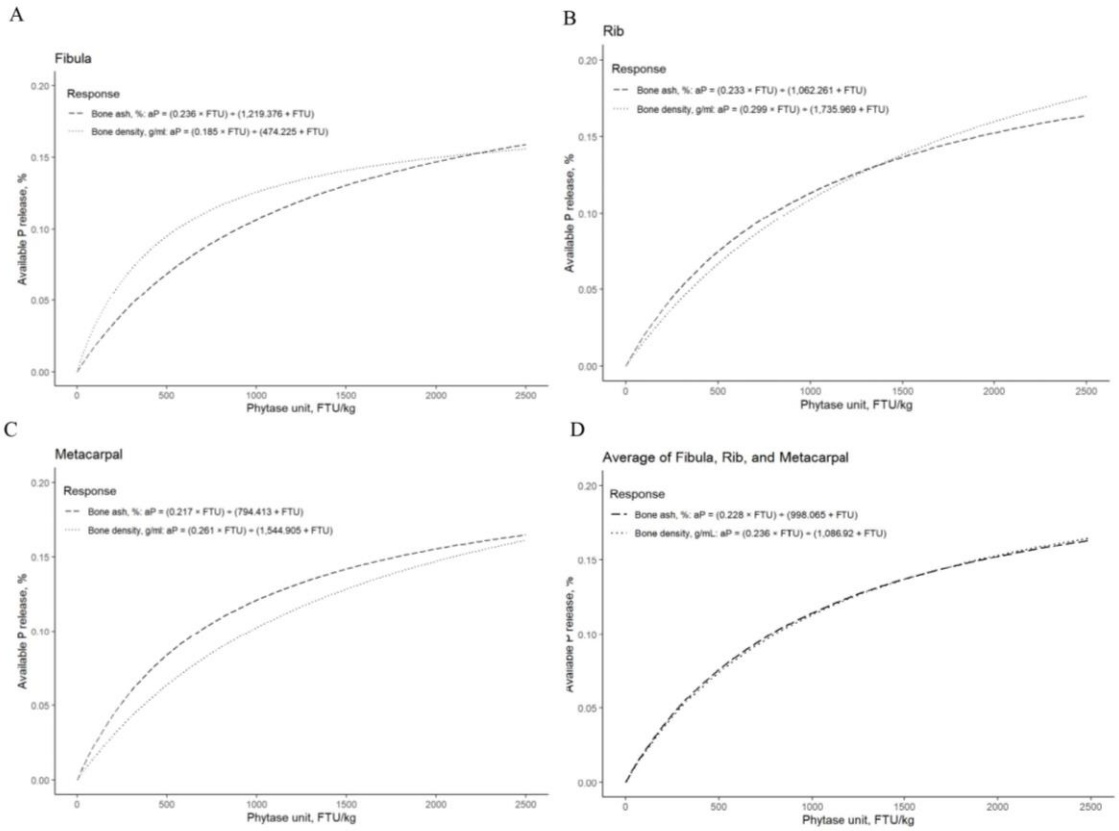

Table 1.1. Analyzed ingredient composition (as-fed basis)

Ingredient	Ca, %	P, %
Limestone[1]	37.06	0.01
Monocalcium P[1]	16.35	19.52
Canola meal[1]	0.60	1.01
Corn[2]	0.07	0.24
Soybean meal[2]	0.56	0.64

[1]Ingredient samples were pooled and analysis was performed by the Kansas State University Soils Lab, Manhattan. Values represent the mean of 6 samples analyzed in duplicate.

[2]Ingredient samples were analyzed by Hubbard Feeds, Beloit, KS.

Table 1.2. Composition of basal mix (as-fed basis)[1]

Item	
Ingredient, %	
Corn	60.90
Soybean meal	29.95
Canola meal	7.61
Sodium chloride	0.61
L-Lysine-HCl	0.30
DL-Methionine	0.10
L-Threonine	0.10
L-Valine	0.01
Trace mineral premix[2]	0.15
Vitamin premix[3]	0.25
Total	100
Calculated analysis	
Standardized ileal digestible (SID) amino acids	
Lys, %	1.24
Ile:lys	64
Leu:lys	130
Met:lys	33
Met and cys:lys	59
Thr:lys	64
Trp:lys	18.7
Val:lys	71
His:lys	43
Total lys, %	1.44
Metabolizable energy, kcal/kg	3,307
Net energy (NE), kcal/kg	2,414
SID lys:NE, g/Mcal	5.14
Crude protein, %	22.6
Ca, %	0.33
P, %	0.42
Available P, %[4]	0.08
STTD P, %[5]	0.17

[1]The basal batch was used as the major ingredient in each experimental diet.

[2]Provided per kg of diet: 110 mg Zn from zinc sulfate; 110 mg Fe from iron sulfate; 33 mg Mn from manganese oxide; 17 mg Cu from copper sulfate; 0.30 mg I from calcium iodate; 0.30 mg Se from sodium selenite.

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

[4]Coefficients for formulation were derived from NRC (1998).

[5]STTD P = Standardized total tract digestible phosphorus.

Table 1.3. Ingredient composition of experimental diets (as-fed basis)[1]

	aP[2]			Phytase[3]				
Ingredient, %	0.11	0.20	0.28	500	1,000	1,500	2,000	2,500
Basal mix	98.55	98.55	98.55	98.55	98.55	98.55	98.55	98.55
Limestone	0.35	0.40	0.45	0.35	0.35	0.35	0.35	0.35
Monocalcium P	0.16	0.58	1.01	0.16	0.16	0.16	0.16	0.16
Sand[4]	0.95	0.48	----	0.92	0.90	0.88	0.86	0.84
Phytase[5]	----	----	----	0.0217	0.0435	0.0652	0.0870	0.1087
Total	100	100	100	100	100	100	100	100
Calculated analysis								
Crude protein, %	22.3	22.3	22.3	22.3	22.3	22.3	22.3	22.3
Ca, %	0.48	0.57	0.66	0.48	0.48	0.48	0.48	0.48
P, %	0.44	0.52	0.60	0.44	0.44	0.44	0.44	0.44
Phytase, FTU/kg	----	----	----	500	1,000	1,500	2,000	2,500
Ca:P ratio	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Analyzed composition[6]								
Ca, %	0.52	0.63	0.74	0.50	0.48	0.54	0.56	0.51
P, %	0.45	0.56	0.65	0.44	0.45	0.46	0.46	0.45
Phytic acid, %[7]	1.11	1.11	1.15	1.07	1.07	1.09	1.14	1.06
Phytase, FTU/kg[8]	----	----	----	519	1,046	1,507	2,036	2,499

[1]Diets were fed for 21 d starting at approximately 12 kg.

[2]Inorganic P was added to the diet by increasing monocalcium P to reach targeted available P (aP) levels.

[3]Smizyme TS G5, Barentz, Woodbury, MN.

[4]Sand was used to equalize hand-add batch including the addition of limestone, monocalcium P, and phytase when blended with the basal mix.

[5]Phytase was analyzed and contained 2,300,000 FTU/kg (Eurofins Scientific Inc., Des Moines, IA).

[6]Complete diet samples were taken during bagging of experimental diets from every fourth bag and pooled into one homogenized sample per dietary treatment. Samples were stored at -20°C until they were submitted for duplicate analysis of Ca and P (Kansas State University Soils Lab, Manhattan).

[7]Two samples of each diet were submitted to Eurofins Scientific Inc. (Des Moines, IA) for analysis of phytic acid using the method outlined in Analytical Biochemistry Vol. 77:536-539 (1977).

[8]Two samples of each diet were submitted to Barentz (Woodbury, MN) for complete phytase analysis using the AOAC official method 2000.12.

Table 1.4. Effects of increasing aP from inorganic P or Smizyme TS G5 2,500 phytase on nursery pig growth performance and bone ash values[1,2]

	aP[3]			Phytase, FTU/kg[4]						Inorganic P, *P* =		Phytase, *P* =	
Item	0.11	0.20	0.28	500	1,000	1,500	2,000	2,500	SEM	Linear	Quadratic	Linear	Quadratic
BW, kg													
d 0	11.9	11.9	11.9	11.9	11.8	11.8	11.9	11.8	0.22	0.824	0.867	0.797	0.883
d 21	21.7	23.9	24.2	24.2	23.6	23.7	24.3	24.2	0.38	< 0.001	0.009	< 0.001	0.004
d 0 to 21													
ADG, g	463	571	584	585	563	563	576	589	14.4	< 0.001	0.005	< 0.001	0.002
ADFI, g	769	862	849	876	840	832	852	866	20.4	0.007	0.033	0.019	0.190
G:F, g/kg	601	663	689	668	670	677	675	680	9.3	< 0.001	0.090	< 0.001	< 0.001
Bone characteristics[5]													
Fibula													
Bone ash, g	0.611	0.749	0.982	0.739	0.808	0.902	0.974	1.002	0.0434	< 0.001	0.381	< 0.001	0.224
Bone ash, %	42.5	45.3	49.1	45.0	46.0	48.5	48.5	48.2	0.83	< 0.001	0.637	< 0.001	0.022
Bone density, g/ml	1.17	1.17	1.22	1.19	1.20	1.21	1.21	1.20	0.011	0.001	0.119	0.008	0.032
P, g	0.107	0.151	0.178	0.130	0.129	0.178	0.173	0.184	0.0102	< 0.001	0.505	< 0.001	0.406
P, %	17.5	20.0	18.2	17.6	16.0	19.9	17.9	18.3	0.86	0.545	0.045	0.201	0.992
Rib													
Bone ash, g	0.730	1.035	1.201	0.894	1.027	1.107	1.171	1.199	0.0504	< 0.001	0.253	< 0.001	0.038
Bone ash, %	44.4	47.9	51.5	47.8	48.5	50.4	51.4	51.0	0.69	< 0.001	0.945	< 0.001	0.005
Bone density, g/ml	1.17	1.17	1.24	1.18	1.20	1.22	1.24	1.22	0.008	< 0.001	0.002	< 0.001	0.027
P, g	0.126	0.204	0.238	0.158	0.176	0.200	0.214	0.216	0.0149	< 0.001	0.175	< 0.001	0.166
P, %	17.4	19.6	19.5	17.7	17.1	17.9	18.3	17.9	0.90	0.065	0.243	0.444	0.989
Metacarpal													
Bone ash, g	1.008	1.290	1.484	1.171	1.283	1.381	1.435	1.523	0.0552	< 0.001	0.520	< 0.001	0.233
Bone ash, %	32.4	34.9	39.0	35.2	36.9	37.8	39.2	38.0	0.94	< 0.001	0.505	< 0.001	0.016
Bone density, g/ml	1.14	1.16	1.18	1.16	1.16	1.17	1.18	1.18	0.005	< 0.001	0.437	< 0.001	0.086
P, g	0.188	0.228	0.253	0.193	0.227	0.233	0.244	0.268	0.0124	< 0.001	0.600	< 0.001	0.994
P, %	18.6	17.6	17.1	16.4	17.8	16.8	17.0	17.7	0.68	0.129	0.778	0.500	0.139

[1]A total of 320 nursery pigs (Line 241 × 600, DNA, Columbus, NE; initially 11.9 kg body weight (BW)) were used in a 21-d growth trial to determine the available P (aP) release curve for Smizyme TS G5 2,500 phytase. There were 5 pigs per pen and 8 replications per treatment.

[2]ADG = average daily gain; ADFI = average daily feed intake; G:F = gain-to-feed ratio.

[3]Inorganic P was added to the diet by increasing monocalcium P.

[4]Smizyme TS G5, Barentz, Woodbury, MN.

[5]One pig per pen (8 pens per treatment) was euthanized and the right fibula, rib, and metacarpal were collected to determine bone density, bone ash weight, percentage bone ash, and bone P. After cleaning, bones were submerged in ultra-purified water under vacuum for 4 h. Weights were then collected, and bone density calculated. For bone ash, bones were placed in a drying oven at 105ºC for 7 d and then ashed in a muffle furnace at 600ºC for 24 h. For bone P, a portion of the residual bone ash was placed in a tube with nitric acid and incubated at 60°C for 6 h. The solution was then diluted using ultra-purified water and P quantity measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Table 1.5. Calculated aP release values based on different response criteria[1,2]

	Phytase, FTU/kg[3]						Probability, *P* =	
Item	500	1,000	1,500	2,000	2,500	SEM[4]	Linear	Quadratic
Performance								
ADG	0.138	0.112	0.112	0.128	0.145	0.0199	< 0.001	0.002
G:F	0.115	0.122	0.138	0.130	0.139	0.0194	< 0.001	< 0.001
Bone characteristics[4]								
Fibula								
Bone ash, %	0.062	0.093	0.161	0.149	0.144	0.0223	< 0.001	0.026
Bone density, g/mL	0.083	0.123	0.173	0.162	0.124	0.0362	0.008	0.033
Rib								
Bone ash, %	0.079	0.100	0.145	0.164	0.154	0.0169	< 0.001	0.004
Bone density, g/mL	0.048	0.107	0.149	0.195	0.144	0.0208	< 0.001	0.041
Metacarpal								
Bone ash, %	0.077	0.123	0.144	0.175	0.146	0.0234	< 0.001	0.017
Bone density, g/mL	0.072	0.090	0.125	0.168	0.150	0.0177	< 0.001	0.071
Average								
Bone ash, %	0.074	0.106	0.149	0.164	0.149	0.0180	< 0.001	0.004
Bone density, g/mL	0.066	0.107	0.150	0.177	0.140	0.0200	< 0.001	0.011

[1]The marginal intake of available P (aP) per day was calculated for each pen using the equation: aP intake/day = [dietary aP% - 0.11% (aP in the basal diet)] × ADFI. A standard curve was then developed for each response criterion using the marginal aP release as the predictor variable. The equation for the standard curve was used to calculate aP release from each pen fed the different phytase dosages based on the observed value for each response criterion.

[2]ADG = average daily gain. G:F = gain-to-feed ratio.

[3] Smizyme TS G5, Barentz, Woodbury, MN.

[4] One pig per pen (8 pens per treatment) was euthanized and the right fibula, rib, and metacarpal were collected to determine bone density and percentage bone ash. After cleaning, bones were submerged in ultra-purified water under vacuum for 4 h. Weights were then collected, and bone density calculated. For bone ash, bones were placed in a drying oven at 105°C for 7 d and then ashed in a muffle furnace at 600°C for 24 h.

[5]Average aP release values generated using data from the right fibula, rib, and metacarpal.

Chapter 2 - Evaluating the effects of benzoic acid on nursery and finishing pig growth performance

ABSTRACT

Three studies were conducted evaluating the use of benzoic acid in swine diets (VevoVitall, DSM Nutritional Products, Parsippany, NJ). In experiment 1, 350 weanling barrows (DNA 200 × 400; initially 5.9 ± 0.04 kg) were allotted to 1 of 5 dietary treatments with 14 pens per treatment. Diets were fed in 3 phases: phase 1 from weaning to d 10, phase 2 from d 10 to 18, and phase 3 from d 18 to 38. Treatment 1 contained no benzoic acid throughout all three phases (weaning to d 42). Treatment 2 included 0.50% benzoic acid throughout all three phases. Treatment 3 contained 0.50% benzoic acid for phase 1 and phase 2, and 0.25% benzoic acid in phase 3. Treatment 4 contained 0.50% benzoic acid in phase 1 and phase 2, and no benzoic acid in phase 3. Treatment 5 contained 0.50% benzoic acid in phase 1, 0.25% benzoic acid in phase 2, and no benzoic acid in phase 3. For the overall period, pigs fed 0.50% in the first two phases and 0.25% benzoic acid in the final phase had greater ($P < 0.05$) average daily gain (ADG) than pigs fed no benzoic acid through all three phases, or pigs fed 0.50% in the first two phases and no benzoic acid in the final phase, with pigs fed the other treatments intermediate. Pigs fed 0.50% in the first two phases and 0.25% benzoic acid in the final phase had improved ($P < 0.05$) gain-to-feed ratio (G:F) compared with pigs fed no benzoic acid throughout all three phases, pigs fed 0.50% in the first two phases and no benzoic acid in the third phase, or pigs fed 0.50%, 0.25%, and no benzoic acid, respectively. For experiment 2, a 101-d trial was conducted using two groups of 1,053 finishing pigs (2,106 total pigs; PIC 337 × 1050; initially 33.3 ± 1.9 kg). Dietary treatments were corn-soybean meal-dried distillers grains with solubles-based with the addition of none, 0.25, or 0.50% benzoic acid. Overall, pigs fed increasing benzoic acid had a tendency

for increased average daily feed intake (ADFI; linear, $P = 0.083$) but decreased G:F (linear, $P < 0.05$). In experiment 3, 2,162 finishing pigs (DNA 600 × PIC 1050; initially 31.4 ± 2.2 kg) were used in a 109-d trial. Dietary treatments were formulated with or without 0.25% benzoic acid. For the overall experimental period, pigs fed benzoic acid had increased ($P < 0.05$) G:F. In summary, feeding benzoic acid elicits improved growth performance when fed throughout the entire nursery period while improved G:F in growing-finishing pigs was observed in one experiment, but not in the other.

INTRODUCTION

As the swine industry continues to reduce the use of feed-grade antimicrobial growth promoters and pharmacological levels of Zn, finding new alternatives that elicit similar responses has become a focal point (Kongsted and McLoughlin, 2023). A specific class of feed additives that has shown positive effects on growth performance and gut health are organic acids (Suiryanrayna and Ramana, 2015). Acidifiers, such as benzoic acid, are suggested to lower the pH of the gastrointestinal tract leading to potential improvements in nutrient digestion, growth performance, and gut microbiota (Torrallardona et al., 2007; Rao et al., 2023). Furthermore, benzoic acid has been shown to exhibit antimicrobial properties within the gastrointestinal tract (Knarreborg et al., 2002). This mechanism is largely driven by the acidic environment inhibiting the growth of pathogens through the accumulation of hydrogen ions. The acids themselves can also disrupt bacteria cell walls (Nguyen et al., 2020).

Due to these benefits, the effects of benzoic acid on growth performance and intestinal morphology have been widely studied (Torrallardona, et al., 2007; Zhai et al., 2017; Warner et al., 2023). Most studies have reported positive responses in growth performance, but only evaluate one level of the acidifier, often in combination with other feeding strategies

(Torrallardona et al., 2007; Zhai et al., 2020; Warner et al., 2023). The few studies that have evaluated multiple benzoic acid feeding levels in nursery diets observed positive responses up to the highest inclusion level (Zhai et al., 2017; Silveria et al., 2018). Despite these findings, previous studies have failed to evaluate the feeding duration throughout the nursery period, thus disregarding an important economical component. Therefore, additional research is needed to further validate benzoic acid feeding strategies throughout the entire nursery period.

Despite the information on benzoic acid inclusion in nursery diets, there has been limited research focusing on the addition of benzoic acid in finishing diets. Recently, a review by Rao et al. (2023) focused on the effects of different feed additives on finishing pig growth performance concluding that acidifiers had the potential to positively impact feed efficiency when compared to other feed additives. However, the challenge with the current research utilizing benzoic acid in finishing diets is the consistency of response. In the feed additive review, implementing acidifiers in finishing diets had an impact on average daily gain (ADG) ranging from -14.9 to 11.4% and an impact on feed efficiency ranging from -9.7 to 11.3% (Rao et al., 2023). More research is needed to understand the consistency in response from feeding finishing pigs diets containing acidifiers. Consequently, the objective of these studies was to evaluate different benzoic acid feeding strategies on nursery pig performance and investigate the effects of benzoic acid supplementation in finishing pig diets.

MATERIALS AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments. Experiment 1 was conducted at the Kansas State University Segregated Early Weaning Facility located in Manhattan, KS. The pigs were housed in two identical barns. Each barn was enclosed and environmentally controlled using mechanical

ventilation. Pens had a metal tri-bar floor, contained a cup waterer, and had a 4-hole, dry self-feeder. Pens (1.2 × 1.2 m) housed 5 pigs which allowed approximately 0.30 m^2/pig. Experiment 2 was conducted in two barns at a commercial research grow-finish site located in southwest Minnesota (New Horizon Farms, Pipestone, MN). Each barn had slatted concrete floors, deep pit manure storage, were naturally ventilated, and double-curtain-sided. Pens (3.0 × 5.5 m) contained a cup waterer and a 5-hole stainless steel dry self-feeder (Thorp Equipment, Thorp, WI). Pigs were allowed approximately 0.6 m^2/pig. Experiment 3 was conducted at a commercial research facility located in southwest Minnesota (Pipestone Nutrition, Edgerton, MN). Pigs were housed in a temperature-controlled wean-to-finish facility. Each pen (6.8 × 2.5 m) contained 1 nipple waterer and a 4-hole dry self-feeder. Pigs were allowed approximately 0.6 m^2/pig. Daily feed additions were recorded for experiment 2 and 3 with a computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN). Pigs were provided *ad libitum* access to the treatment diets and water throughout all three experiments.

Animals and diets

For experiment 1, a total of 350 weanling barrows (DNA 200 × 400; initially 5.9 ± 0.04 kg) were randomly assigned to pens and pens were allotted to 1 of 5 dietary treatments with 14 replications per treatment. Diets were fed in 3 phases: phase 1 from weaning to d 10, phase 2 from d 10 to 18, and phase 3 from d 18 to 38. Dietary treatments were formulated to provide 0, 0.25, or 0.50% benzoic acid (minimum 99.5% pure benzoic acid; VevoVitall, DSM Nutritional Products, Parsippany, NJ) added at the expense of corn (Table 2.1). Treatment 1 served as the control and contained no benzoic acid throughout all three phases. Treatment 2 included 0.50% benzoic acid throughout all three phases. Treatment 3 contained 0.50% benzoic acid for phase 1 and phase 2, then 0.25% benzoic acid in phase 3. Treatment 4 contained 0.50% benzoic acid in

phase 1 and phase 2, and no benzoic acid in phase 3. Treatment 5 contained 0.50% benzoic acid in phase 1, 0.25% benzoic acid in phase 2, and no benzoic acid in phase 3. For both the phase 1 and phase 2 diets, a single batch of a base diet was manufactured (Hubbard Feeds, Beloit, KS), then benzoic acid additions were mixed in at the Kansas State University O.H. Kruse Feed Technology Innovation Center, Manhattan, KS. For phase 3, complete diets were manufactured (Hubbard Feeds, Beloit, KS). The diets were fed in meal form and pig weights and feed disappearance were measured on d 0, 10, 18, 24, and 38 to determine ADG, average daily feed intake (ADFI), and gain-to-feed ratio (G:F). Feces were collected on d 10 and 24 from 3 pigs per pen to determine fecal dry matter (DM). Samples were dried at 55°C for 48 h and loss of weight was used to determine percentage fecal DM.

In experiment 2, a 101-d growth trial was conducted using two groups of 1,053 finishing pigs (2,106 total pigs; PIC 337 × 1050; initially 33.3 ± 1.91 kg). Pens of pigs (27 pigs per pen), with a similar number of barrows and gilts per pen, were randomly assigned to 1 of 3 dietary treatments in a completely randomized design with 13 replications per treatment in each barn for a total of 26 replications. Dietary treatments were corn-soybean meal-dried distillers grains with solubles-based with the addition of none, 0.25, or 0.50% benzoic acid. Diets were fed in 4 phases from 34 to 50, 50 to 75, 75 to 100, and 100 to 132 kg body weight. All treatments were formulated to meet or exceed NRC (2012) requirements for finishing pigs for their appropriate weight ranges (Table 2.2). All diets were manufactured at New Horizon Farms Feed Mill (Pipestone, MN). Every two weeks, pens of pigs were weighed and feed disappearance was measured to determine ADG, ADFI, and G:F.

Three pigs per pen were weighed and marketed two weeks prior to the end of the experiment. These pigs were included in growth performance data but were not evaluated for

carcass characteristics. At the completion of the experiment, the remaining pens of pigs were weighed and marketed. Pigs were transported to a U.S. Department of Agriculture-inspected packing plant (JSB Swift, Worthington, MN). Carcass data were collected including hot carcass weight (HCW), loin depth, and backfat. Percentage lean was calculated using a proprietary equation from the plant. Carcass yield was calculated using the pen average HCW divided by the pen average final live weight.

In experiment 3, a total of 2,162 finishing pigs (DNA 600 × PIC 1050; initially 31.4 ± 0.47 kg) were used in a 109-d trial. Dietary treatments were formulated in 2 × 2 factorial with main effects of soybean meal source and benzoic acid. The main effect of soybean meal source was included to accomplish a separate objective and will not be discussed further as there were no interactions with benzoic acid inclusion. Dietary treatments for the main effect of benzoic acid were formulated with or without 0.25% benzoic acid. There were 27 or 28 pigs per pen and 40 pens per benzoic acid treatment. On d 0, pens were blocked by location in the barn and randomly allotted to 1 of 2 benzoic acid treatment levels. A similar number of barrows and gilts were placed in each pen. Experimental diets were fed in 6 different phases. Pigs were fed by a feed budget with phases 1, 2, 3, 4, and 5 provided at 19, 42, 49, 50, and 45 kg per pig, respectively. Phase 6 was provided for the remainder of the study until pigs were marketed. Pens of pigs and feed disappearance were measured approximately every 14 d to determine ADG, ADFI, and G:F. All nutrients were formulated to meet or exceed NRC (2012) requirement estimates (Table 2.3). All diets were corn-soybean meal-based and were fed in meal form. Diets were manufactured at the Spronk Brothers Feed Mill (Edgerton, MN).

On d 88 and 96, eight of the heaviest pigs per pen were weighed individually and transported to a commercial packing plant (WholeStone Farms, Fremont, NE) for processing and

carcass data collection. The remaining pigs were marketed at the conclusion of the trial on d 109 and transported to WholeStone Farms for carcass data collection. A fat sample was taken from the belly of one barrow per pen per marketing event and included all three layers of fat. Analysis of iodine value was conducted using near-infrared spectroscopy at WholeStone Farms.

Statistical Analysis

For experiment 1, data were analyzed as a completely randomized design with pen serving as the experimental unit, treatment as a fixed effect, and barn as a random effect. Data were analyzed using R Studio (Version 3.5.2, R Core Team, Vienna, Austria). Contrasts were used to test for the main effects of the different benzoic acid feeding levels (0, 0.25, and 0.50%), within the three phases. Overall performance data were analyzed as a one-way ANOVA using the lmer function from the lme4 package. Differences for treatments demonstrating a significant source of variation were determined through pairwise comparisons using the Tukey-Kramer multiplicity adjustment to control for type I error. Similarly, contrasts were used to test for the main effects of treatment, day, and interaction between treatment and day of different benzoic feeding levels on fecal DM.

For experiment 2, data were analyzed as a completely randomized design with pen as the experimental unit and treatment as the fixed effect. Data were analyzed as a one-way ANOVA using the lmer function from the lme4 package in R (version 4.1.1 (2021-08-10), R Foundation for Statistical Computing, Vienna, Austria). Contrasts were used to test the main effect of benzoic acid level (0, 0.25, and 0.50%). Similarly, contrasts were used to analyze carcass characteristics including backfat, loin depth, and percentage lean with HCW serving as a covariate.

For experiment 3, data were analyzed using the GLIMMIX procedure of SAS OnDemand for Academics (SAS Institute, Inc., Cary, NC) in a randomized complete block design with pen as the experimental unit and location as the blocking factor. Treatments were considered a fixed effect and block as a random effect. The main effect of benzoic acid (0 vs. 0.25%) was analyzed. For all three experiments, results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 > P \leq 0.10$.

RESULTS

Experiment 1

From d 0 to 10 (phase 1), pigs fed 0.50% benzoic acid had increased ($P \leq 0.05$) ADG, G:F, and heavier d 10 body weight (BW) than those fed the control diet (Table 2.4). From d 10 to 18 (phase 2), pigs fed 0.50% benzoic acid had increased ($P < 0.01$) ADG compared to pigs fed either none or 0.25% benzoic acid, while pigs fed 0.25% benzoic acid had decreased ($P < 0.001$) G:F compared to pigs fed none or 0.50% benzoic acid. There was a significant increase in ADFI for pigs fed 0.25% ($P = 0.033$) benzoic acid and a marginally significant increase in ADFI for pigs fed 0.50% ($P = 0.069$) benzoic acid in phase 2 compared to pigs fed no benzoic acid; however, they did not differ from each other. Pigs fed 0.50% benzoic acid had increased ($P = 0.012$) d 18 BW compared to pigs fed no benzoic acid, while pigs fed 0.25% benzoic acid were intermediate. From d 18 to 38 (phase 3), pigs fed 0.50 or 0.25% benzoic acid had increased ($P < 0.01$) ADG and ADFI compared with pigs fed no benzoic acid. Additionally, pigs fed 0.25% benzoic acid in phase 3 had improved ($P < 0.05$) G:F compared to pigs fed none or 0.50% benzoic acid.

For the overall experimental period (d 0 to 38), pigs fed 0.50% benzoic acid in the first two phases and 0.25% benzoic acid in the final phase had a greater ($P < 0.05$) ADG than pigs fed

no benzoic acid through all three phases and pigs fed 0.50% benzoic acid in the first two phases with no benzoic acid in the final phase, while pigs fed the other treatments were intermediate. Pigs fed 0.50% in phase 1 and 2 and 0.25% benzoic acid in the final phase had increased ($P < 0.05$) G:F compared with pigs fed no benzoic acid throughout all three phases, pigs fed 0.50% in the first two phases and no benzoic acid in the third phase, and pigs fed 0.50%, 0.25%, and no benzoic acid, respectively. There was also evidence for differences ($P < 0.01$) in d 38 BW with pigs fed 0.50% benzoic acid in the first two phases and 0.25% benzoic acid in the third phase having increased ($P < 0.01$) BW compared with pigs fed no benzoic acid throughout all three phases and pigs fed 0.50% benzoic acid in the first two phases with no benzoic acid in the final phase. There was no evidence ($P > 0.10$) of an interaction between treatment and day for fecal DM. Furthermore, there was no evidence of a main effect of treatment ($P > 0.10$) for fecal DM. However, there was evidence for a main effect of day ($P < 0.001$) with greater fecal DM on d 10 vs. 18.

Experiment 2

In the grower period (d 0 to 44), there was no evidence of differences ($P > 0.10$) for any growth response criteria (Table 2.5). For the finisher period (d 44 to 101), increasing benzoic acid tended to increase ADFI (linear, $P = 0.053$) but decrease G:F (linear, $P = 0.002$). There was no evidence for differences ($P > 0.10$) in ADG. Similarly, for the overall experimental period (d 0 to 101), pigs fed increasing benzoic acid had a tendency for increased ADFI (linear, $P = 0.083$) and decreased G:F (linear, $P = 0.011$). There was no evidence of difference in ADG ($P > 0.10$) for the overall experimental period. Furthermore, there was no evidence of differences ($P > 0.10$) in grower BW (d 44) or final BW (d 101).

For carcass characteristics, no evidence of difference ($P > 0.10$) was observed for any criteria including HCW, carcass yield, backfat, loin depth, or percentage lean due to increasing benzoic acid.

Experiment 3

From d 0 to 51, pigs fed diets without benzoic acid had greater ($P \leq 0.01$) ADG and ADFI compared to pigs fed diets containing benzoic acid (Table 2.6). Pigs fed diets containing benzoic acid had increased ($P < 0.001$) G:F compared to pigs fed diets without benzoic acid. From d 51 to 109, there was a tendency for an increase ($P = 0.06$) in ADG in pigs fed diets containing benzoic acid compared to those fed diets without benzoic acid. There were no effects ($P > 0.10$) of benzoic acid on ADFI or G:F during this period. Overall (d 0 to 109), pigs fed benzoic acid had decreased ($P = 0.02$) ADFI, but similar ($P > 0.10$) ADG. As a result, pigs fed benzoic acid had improved ($P = 0.01$) G:F compared to pigs fed diets without benzoic acid. Carcass characteristics and iodine value were not impacted ($P > 0.10$) by benzoic acid treatment.

DISCUSSION

As the swine industry decreases the use of antimicrobial growth promoters and pharmacological levels of Zn, researchers have begun to investigate multiple feed additives including acidifiers, prebiotics, probiotics, phytogenics, nucleotides, and direct-fed microbials in order to maintain pig health and growth performance (Liu et al., 2018). The growing interest in acidifiers is largely driven by the proposed multi-mechanistic effects driven by the lowered gastric pH. These effects are outlined by Liu et al. (2018) and include 1) increased pepsin activity, 2) inhibition of pathogenic bacteria, and 3) improved nutrient digestibility.

Benzoic acid is an aromatic carboxylic acid and is classified as a weak organic acid. When added at 0.50% in a complete swine diet, benzoic acid has the ability to reduce the

calculated acid-binding capacity-4 by approximately 30 mEq/kg (Warner et al., 2023). Although benzoic acid tends to be less acidic than other organic acids, such as formic acid, it has been shown to successfully decrease the pH of stomach contents when fed at levels ranging from 0.20 to 0.75% (Chen et al., 2017; Silveira et al., 2018). In addition to acidification of the gastrointestinal tract, benzoic acid has been shown to reduce urine pH through excreting the metabolite, hippuric acid.

Weaning is a stressful event in a pig's life and typically occurs during a critical window of immune and intestinal development (Moeser et al., 2017). During this time, weaned pigs exhibit reduced stomach acid secretions which do not typically peak until 56 d of age (Yen, 2001; Pluske, 2016). Thus, a pig is challenged with a multitude of factors including changes in environment, diet, and physiological development at the time of weaning. Therefore, by the addition of an acidifier in nursery diets, there is a potential for a reduction in pH in the digestive tract. This reduction in pH has the potential to assist a young pig through antimicrobial effects, increased nutrient digestion, and improved intestinal health, ultimately yielding improved growth performance (Liu et al., 2018; Tugnoli et al., 2020). Many studies utilizing benzoic acid in nursery diets only evaluate one level of the acidifier often in combination with other feeding strategies (Guggenbuhl et al., 2007; Torrallardona et al., 2007; Zhai et al., 2020; Warner et al., 2023). These studies all found significant improvements in growth performance including increased ADG and G:F with 0.50% benzoic acid added to the diet. Furthermore, the few studies that have evaluated multiple benzoic acid feeding levels in nursery diets observed positive responses up to the highest levels of inclusion, 0.50 and 0.75% (Zhai et al., 2017; Silveria et al., 2018). Despite previous literature evaluating growth performance of nursery pigs supplemented

with benzoic acid, there is currently no research investigating feeding duration and level throughout the nursery period.

A review by Kil et al. (2011) outlined the results of multiple previous studies utilizing benzoic acid in the nursery period and found a positive response during the first 2 wk post-weaning. However, according to experiment 1, it appears that feeding 0.50% benzoic acid in all three phases or 0.50% benzoic acid for the first two phases and 0.25% in the third phase will result in the best growth performance for nursery pigs. The reduction in performance reported when benzoic acid was removed from the diet both when transitioning from phase 1 to phase 2 and when transitioning from phase 2 to phase 3 is a unique finding of this study. One potential explanation for this decrease in performance is a change in feed palatability when benzoic acid is removed from the diet. A study by Partanen et al. (2002) reported that pigs preferred diets containing sodium benzoate compared to other organic acids such as formic and lactic acid. However, in experiment 1, there was no decrease in feed intake witnessed when benzoic acid was reduced in the diet in phase 2. Therefore, the reduction in ADG may be attributed to shifts in gastrointestinal pH or digestibility when benzoic acid levels change. Furthermore, since pigs were 18 d post-weaning and approximately 10.5 kg BW when this change from phase 2 to phase 3 occurred, this might indicate their gastrointestinal system had not matured enough in physiological development to handle a less complex diet. Ultimately, further research is needed to truly understand the mechanism behind this decrease in growth performance.

Similar to the nursery period, implementing the optimal level and duration of benzoic acid in growing-finishing diets presents unique challenges. A review by Rao et al. (2023) focusing on the effects of feed additives on finishing pig growth performance showed acidifiers had a potential positive impact on feed efficiency when compared to other feed additives.

However, the challenge with the current research utilizing benzoic acid in finishing diets is the consistency of response. In the feed additive review, implementing acidifiers in finishing diets had a wide impact on growth performance with effects on ADG ranging from -14.9 to 11.4% and feed efficiency ranging from -9.7 to 11.3% (Rao et al., 2023). The data in experiment 2 investigating the effects of feeding increasing levels of benzoic acid suggests that feeding benzoic acid in the grow-finish period had no effect on ADG, but tended to increase ADFI and decreased G:F by 1.7%. These data contradict past studies showing a positive (Zhai et al., 2017) or no response (Cho et al., 2014; O'Meara et al., 2020) to benzoic acid supplementation in the growing-finishing period. The consistency of response to benzoic acid supplementation has proven to be more variable during the grow-finish production phase compared to the nursery period across past literature. This inconsistency in response was demonstrated further in experiment 3 where G:F improved by 1.1% when 0.25% benzoic acid was added to the diet.

In summary, these data suggest that feeding benzoic acid for the first 38 d post-weaning improves ADG and G:F. However, when benzoic acid was removed from the nursery diet, pigs experienced a reduction in performance and ultimately had similar performance to pigs fed no benzoic throughout the entire experimental period. In this study, feeding 0.50% benzoic acid in all three phases or 0.50% benzoic acid for the first two phases and 0.25% in the third phase resulted in the best growth performance throughout the nursery period. Furthermore, the two finisher trials conducted demonstrate the inconsistency of response to benzoic acid supplementation in finishing diets. One trial suggests that feeding benzoic acid in the grow-finish period had no impact on ADG, but tended to increase ADFI and worsen G:F while the other study found that additions of benzoic acid improved feed efficiency. Overall, further research is warranted to better understand under what conditions a positive response might repeatedly be

observed in the growing-finishing period as well as understanding the carry-over effect of benzoic acid supplementation from the nursery into the finishing period.

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Table 2.1. Diet composition (as-fed basis), Exp. 1[1]

Item, %	Phase 1	Phase 2	Phase 3
Ingredient, %			
Corn	44.90	50.15	64.25
Soybean meal	17.40	23.75	31.80
Spray-dried whey	10.00	---	---
Whey permeate	10.00	10.00	---
Dried distillers grains with solubles	5.00	7.50	---
Fish meal	2.50	---	---
Fermented soybean meal[2]	4.00	3.85	---
Spray-dried bovine plasma	2.00	---	---
Choice white grease	1.00	1.00	---
Monocalcium P	0.80	1.00	1.00
Calcium carbonate	0.45	0.45	0.90
Zinc oxide	0.40	0.25	---
Sodium chloride	0.30	0.50	0.60
Vitamin premix with phytase[3]	0.25	0.25	0.25
Trace mineral premix[4]	0.15	0.15	0.15
L-Lys-HCL	0.40	0.55	0.50
DL-Met	0.19	0.22	0.21
L-Thr	0.17	0.22	0.21
L-Trp	0.02	0.04	0.04
L-Val	0.08	0.12	0.13
Benzoic acid[5]	+/-	+/-	+/-
Total	100	100	100
Calculated analysis			
Standardized ileal digestible (SID) amino acids, %			
Lys, %	1.35	1.35	1.35
Ile:Lys	57	57	56
Leu:Lys	120	118	115
Met:Lys	36	37	36
Met and Cys:Lys	59	58	58
Thr:Lys	64	63	63
Trp:Lys	19.2	19.3	19.4
Val:Lys	70	70	70
Total Lys, %	1.51	1.51	1.50
ME, kcal/kg	3,401	3,373	3,278
NE, kcal/kg	2,542	2,502	2,418
SID Lys:ME, g/Mcal	3.97	4.00	4.12
SID Lys:NE, g/Mcal	5.31	5.40	5.58
Crude protein, %	21.5	21.5	21.3
Ca, %	0.66	0.54	0.71
P, %	0.66	0.61	0.61
STTD P, %[6]	0.58	0.51	0.47

[1]Phase 1 diets were fed from 5.9 kg to 7.4 kg, phase 2 diets were fed from 7.4 kg to 10.5 kg, and phase 3 diets were fed from 10.5 kg to 21.0 kg.

[2] MEPro, Prairie Aquatech, Brookings, SD.

[3]Provided per kg of diet: 4,134 IU vitamin A; 1,653 IU vitamin D; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin B12; 50 mg niacin; 28 mg pantothenic acid; 8 mg riboflavin. Ronozyme HiPhos GT, DSM Nutritional Products, Parsippany, NJ was included at 1,250 FTU/kg to provide an estimated release of 0.14% STTD P for all diets.

[4]Provided per kg of diet: 110 mg Zn from zinc sulfate; 110 mg Fe from iron sulfate; 33 mg Mn from manganese oxide; 17 mg Cu from copper sulfate; 0.30 mg I from calcium iodate; 0.30 mg Se from sodium selenite.

[5]Benzoic acid (VevoVitall, DSM Nutritional Products, Parsippany, NJ) at none, 0.25, or 0.50% of the diet was included at the expense of corn.

[6]STTD P = Standardized total tract digestible phosphorus.

Table 2.2. Diet composition (as-fed basis), Exp. 2[1]

Item	Phase 1	Phase 2	Phase 3	Phase 4
Ingredients, %				
Corn	61.09	67.61	71.90	82.91
Soybean meal	26.63	20.40	16.29	15.50
Dried distillers grains with solubles	10.00	10.00	10.00	---
Limestone	1.08	1.00	1.00	0.80
Monocalcium P	0.40	0.25	0.10	0.10
Sodium chloride	0.35	0.35	0.35	0.35
L-Lys-HCl	0.28	0.24	0.22	0.18
DL-Met	0.01	---	---	---
Thr[2]	0.04	0.03	0.01	0.04
Vitamin-trace mineral premix[3]	0.10	0.10	0.10	0.10
Phytase[4]	0.03	0.03	0.03	0.03
Benzoic acid[5]	+/-	+/-	+/-	+/-
Total	100	100	100	100
Calculated analysis				
Standardized ileal digestible (SID) amino acids, %				
Lys	1.08	0.90	0.78	0.70
Ile:Lys	67	69	71	69
Leu:Lys	151	165	178	169
Met:Lys	28	30	32	31
Met and Cys:Lys	55	59	63	62
Thr:Lys	61	62	63	65
Trp:Lys	19	19	19	19
Val:Lys	75	79	83	80
His:Lys	45	47	50	49
Total Lys, %	1.24	1.04	0.91	0.80
ME, kcal/kg	3,261	3,261	3,274	3,336
NE, kcal/kg	2,438	2,480	2,506	2,546
SID Lys:ME, g/Mcal	3.31	2.76	2.38	2.10
SID Lys:NE, g/Mcal	4.43	3.63	3.11	2.75
Crude protein, %	20.9	18.4	16.7	14.4

Ca, %	0.56	0.49	0.45	0.37
STTD P, %[6]	0.41	0.36	0.32	0.28

[1]Phases 1, 2, 3, and 4 were fed from approximately 33 to 49.9 kg, 49.9 to 74.8 kg, 74.8 to 99.8 kg and 99.8 kg to market, respectively.

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

[3]The vitamin and trace mineral premix provided per kg of diet: 5,292 IU vitamin A; 1,323 IU vitamin D; 26.5 IU vitamin E; 2.7 mg vitamin K; 0.02 mg vitamin B12; 49.6 mg niacin; 16.5 mg pantothenic acid; 5.0 mg riboflavin; 16.5 mg Cu; 0.3 mg I; 110.0 mg Fe; 33.1 mg Mn; 0.3 mg Se; 110.0 mg Zn.

[4]Optiphos Plus (Huevepharma, Sofia, Bulgaria) was included at 750 FTU/kg to provide an estimated release of 0.13% STTD P for all diets.

[5]Benzoic acid (VevoVitall, DSM Nutritional Products, Parsippany, NJ) at none, 0.25, and 0.50% of the diet was included at the expense of corn.

[6]STTD P = Standardized total tract digestible phosphorus.

Table 2.3. Diet composition (as-fed basis), Exp. 3[1]

	Phase 1		Phase 2		Phase 3		Phase 4		Phase 5		Phase 6	
Ingredient, %	SBM	EESBM	SBM	EESBM	SBM	EESBM	SBM	EESBM	SBM	EESBM	SBM	EESBM
Corn	64.55	60.04	68.95	65.56	73.98	71.70	77.33	75.66	78.24	76.34	85.72	84.27
Soybean meal	22.60	---	18.35	---	13.50	---	10.30	---	9.65	---	12.35	---
EESBM[2]	---	27.04	---	21.67	---	15.74	---	11.94	---	11.55	---	13.75
DDGS	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	---	---
Limestone	0.88	0.88	0.88	0.88	0.85	0.85	0.83	0.83	0.78	0.78	0.68	0.68
Monocalcium phosphate	0.34	0.35	0.32	0.33	0.28	0.28	0.22	0.23	0.13	0.13	0.32	0.33
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Lys-HCl	0.55	0.58	0.51	0.54	0.48	0.50	0.46	0.47	0.40	0.40	0.24	0.26
DL-Met	0.16	0.19	0.12	0.14	0.08	0.09	0.05	0.06	0.02	0.03	0.00	0.00
L-Trp	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02	---	0.01
Thr[3]	0.24	0.24	0.21	0.21	0.19	0.19	0.16	0.16	0.14	0.13	0.08	0.08
L-Val	0.04	0.03	0.02	0.02	---	---	---	---	---	---	---	---
Vitamin and trace mineral premix	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Copper chloride	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Phytase[4]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Benzoic acid[5]	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Total	100	100	100	100	100	100	100	100	100	100	100	100
Calculated analysis												
Standardized ileal digestible (SID) amino acids, %												
Lys	1.20	1.27	1.07	1.12	0.93	0.95	0.83	0.85	0.77	0.79	0.67	0.69
Ile:Lys	55	55	55	55	55	55	55	55	58	58	64	63
Leu:Lys	128	123	134	130	143	139	151	148	160	158	166	160
Met and Cys:Lys	58	58	58	58	58	58	58	58	58	58	60	58
Thr:Lys	63	63	63	63	64	64	64	64	65	65	66	66
Trp:Lys	17	17	17	17	17	17	17	17	17	17	17	17
Val:Lys	65	65	65	65	65	65	66	66	70	70	76	75

ME, kcal/kg	3,186	3,366	3,208	3,353	3,232	3,339	3,252	3,333	3,258	3,336	3,280	3,375
NE, kcal/kg	2,458	2,542	2,480	2,548	2,506	2,557	2,524	2,562	2,531	2,567	2,545	2,592
SID Lys:ME, g/Mcal	3.76	3.76	3.33	3.33	2.85	2.85	2.54	2.54	2.01	2.01	2.00	2.01
SID Lys:NE, g/Mcal	4.88	5.00	4.31	4.40	3.71	3.72	3.29	3.32	3.04	3.07	2.63	2.66
Crude protein, %	19.02	19.57	17.34	17.76	15.42	15.66	14.16	14.31	13.90	14.17	13.01	13.00
Ca, %	0.60	0.60	0.58	0.58	0.55	0.55	0.52	0.52	0.48	0.48	0.48	0.48
STTD P, %	0.37	0.37	0.36	0.36	0.34	0.34	0.32	0.32	0.30	0.30	0.31	0.31

[1]Pigs were fed on a feed budget with phase 1, 2, 3, 4, and 5 provided at 19, 42, 49, 50, and 45 kg per pig, respectively. Phase 6 was provided for the remainder of the study.

[2]Lester Feed and Grain (Lester, IA).

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

[4]Quantum Blue 5P (AB Vista, Marlborough, UK) was included at 2,000 FTU/kg to provide an estimated release of 0.11% STTD P for all diets.

[5]VevoVitall (DSM Products, Parsippany, NJ) was added at 0.25%.

SBM = soybean meal. EESBM = extruded-expelled soybean meal. DDGS = dried distillers grains with solubles.

Table 2.4. Effects of benzoic acid feeding strategy on nursery pig performance, Exp. 1[1,2]

	Benzoic acid, %[3]							
Phase 1	0	0.50	0.50	0.50	0.50			
Phase 2	0	0.50	0.50	0.50	0.25		Contrast, *P* =	
Phase 3	0	0.50	0.25	0	0	SEM	0 vs 0.5%	0 vs 0.25%	0.25 vs 0.50%
BW, kg								
d 0	5.9	5.9	5.9	5.9	5.9	0.04	0.785	---	---
d 10	7.3	7.5	7.4	7.4	7.6	0.09	0.040	---	---
d 18	10.2	10.7	10.6	10.5	10.4	0.14	0.012	0.186	0.340
d 38	20.4^{bc}	21.6^{ab}	21.9^{a}	20.2^{c}	20.8^{abc}	0.36	0.003	< 0.001	0.553
d 0 to 10 (Phase 1)									
ADG, g	136	164	147	150	172	10.0	0.034	---	---
ADFI, g	164	185	176	171	193	10.5	0.144	---	---
G:F, g/kg	821	891	839	878	896	27.3	0.045	---	---
d 10 to 18 (Phase 2)									
ADG, g	363	395	401	382	350	10.0	0.009	0.333	< 0.001
ADFI, g	454	485	483	472	492	12.5	0.069	0.033	0.412
G:F, g/kg	806	816	832	811	712	20.0	0.483	< 0.001	< 0.001
d 18 to 38 (Phase 3)									
ADG, g	510	544	564	485	515	14.7	0.003	< 0.001	0.233
ADFI, g	771	825	821	754	784	24.4	0.004	0.008	0.869
G:F, g/kg	662	660	687	644	658	7.1	0.504	< 0.001	0.009
d 0 to 38 (Overall)									
ADG, g	380^{bc}	410^{ab}	417^{a}	374^{c}	390^{abc}	10.1	---	---	---
ADFI, g	543	581	575	539	567	16.4	---	---	---
G:F, g/kg	700^{b}	706^{ab}	724^{a}	694^{b}	689^{b}	6.2	---	---	---
Fecal DM, %[4]									

d 10	26.32	26.62	24.65	26.18	26.68	0.675	0.739	---	---
d 18	23.58	24.02	23.54	24.51	24.57	0.675	0.450	0.173	0.356

[1]A total of 350 weanling barrows (DNA 200 × 400; initially 5.9 ± 0.04 kg) approximately 21 days of age were used in a 38-d experiment with 5 pigs per pen and 14 pens per treatment.

[2]Different superscript letters within the same row reflect dietary treatment differences ($P \leq 0.05$).

[3]VevoVitall, DSM Nutritional Products, Parsippany, NJ.

[4]Feces from three piglets from each pen were pooled, weighed, and dried to measure fecal dry matter. Treatment × day, $P = 0.679$; Treatment, $P = 0.199$; Day, $P < 0.001$.

Table 2.5. Effects of increasing benzoic acid on grow-finish pigs growth performance and carcass characteristics, Exp. 2[1]

	Benzoic acid, %[2]				*P* =	
Item	0	0.25	0.50	SEM	Linear	Quadratic
BW, kg						
Initial (d 0)	33.3	33.4	33.3	1.91	0.996	0.900
Grower (d 44)	70.3	70.4	71.0	1.79	0.574	0.774
Final (d 101)	125.4	125.7	125.9	1.14	0.707	0.977
Grower (d 0 to 44)						
ADG, kg	0.84	0.84	0.86	0.011	0.389	0.597
ADFI, kg	2.02	2.02	2.07	0.054	0.188	0.588
G:F, g/kg	418	416	414	7.2	0.380	0.983
Finisher (d 44 to 101)						
ADG, kg	0.99	0.99	0.98	0.043	0.616	0.868
ADFI, kg	3.05	3.05	3.11	0.132	0.053	0.340
G:F, g/kg	325	323	317	1.8	0.002	0.303
Overall						
ADG, kg	0.92	0.92	0.93	0.026	0.777	0.630
ADFI, kg	2.59	2.59	2.64	0.085	0.083	0.404
G:F, g/kg	357	355	351	2.2	0.011	0.472
Carcass characteristics						
HCW, kg	92.1	92.5	93.1	0.73	0.254	0.956
Carcass yield, %	72.4	72.5	72.7	0.24	0.359	0.828
Lean, %[3]	56.5	56.4	56.4	0.24	0.745	0.490
Backfat, mm[3]	16.1	16.1	16.0	0.34	0.862	0.591
Loin depth, mm[3]	61.9	61.4	61.3	0.37	0.134	0.487

[1]A total of 2,106 pigs (PIC 337 × 1050; initially 33.3 ± 1.91 kg) were used in two groups with 27 pigs per pen and 26 replicates per treatment.

[2] VevoVitall, DSM Nutritional Products, Parsippany, NJ.

[3]Adjusted using HCW as covariate.

Table 2.6. Main effect of benzoic acid on growth performance, carcass characteristics, and carcass iodine value, Exp. 3[1]

	Benzoic acid[2]			
Item	None	0.25%	SEM	*P* =
BW, kg				
Initial (d 0)	31.4	31.3	0.47	0.85
Grower (d 51)	78.6	77.2	0.71	0.03
Final (d 109)	123.5	122.8	0.74	0.39
Grower (d 0 to 51)				
ADG, kg	0.92	0.90	0.08	0.01
ADFI, kg	2.03	1.91	0.02	< 0.001
G:F, g/kg	456	470	2.7	< 0.001
Finisher (d 51 to 109)				
ADG, kg	0.92	0.94	0.01	0.06
ADFI, kg	2.87	2.91	0.02	0.14
G:F, g/kg	322	324	1.6	0.38
Overall (d 0 to 109)				
ADG, kg	0.92	0.92	0.01	0.37
ADFI, kg	2.43	2.38	0.02	0.02
G:F, g/kg	380	385	1.5	0.01
Carcass characteristics				
HCW, kg	91.5	90.9	0.67	0.18
Yield, %	73.51	73.47	0.01	0.68
Iodine value	70.25	70.52	0.27	0.43

[1]A total of 2,162 pigs (DNA 600 × PIC 1050; initially 31.4 ± 0.47 kg) were used with 27 to 28 pigs per pen and 40 replications per benzoic acid treatment for a 109-day trial.

[2]VevoVitall (DSM Products, Parsippany, NJ) was added at 0.25%.

SBM = soybean meal. EESBM = extruded-expelled soybean meal.

Chapter 3 - Evaluating increasing levels of sodium diformate in diets for nursery and finishing pigs

ABSTRACT

Two studies were conducted to evaluate the effects of sodium diformate (Formi NDF, ADDCON Nordic AS, Porsgrunn, Norway) in swine diets. For experiment 1, 360 barrows (DNA 200 × 400; initially 5.9 ± 0.06 kg) were used in a 38-d study. At weaning, pigs were randomly assigned to pens with 5 pigs per pen. Each pen was allocated to 1 of 6 treatments with 12 pens per treatment. Treatments were formulated to provide none, 0.40, 0.60, 0.80, 1.00, or 1.20% sodium diformate added at the expense of corn. Diets were fed in 3 phases: phase 1 from weaning to d 9, phase 2 from d 9 to 24, and phase 3 from d 24 to 38. From d 0 to 24 (phase 1 and 2), increasing sodium diformate increased (linear, $P = 0.001$) gain-to-feed (G:F). However, sodium diformate did not affect ($P > 0.10$) average daily gain (ADG) or average daily feed intake (ADFI). From d 24 to 38 (phase 3) and overall (d 0 to 38), there was no evidence of differences ($P > 0.10$) due to increasing sodium diformate for any growth response criteria. There was no evidence for differences ($P > 0.10$) in fecal dry matter (DM) on d 9. However, fecal DM decreased (linear, $P < 0.05$; quadratic, $P = 0.097$) as sodium diformate increased on d 24. In experiment 2, 2,200 pigs [Duroc sire (PIC 800 or DNA 600) × PIC Camborough; initially 24.2 × 0.30 kg] were used in a 117-d growth trial. Pens of pigs (25 pigs per pen) were randomly assigned to 1 of 4 treatments with 22 pens per treatment. Treatments were formulated with additions of none, 0.25, 0.50, or 0.75% sodium diformate. Diets were fed in 6 phases from 24 to 141 kg. For period 1 (d 0 to 32), increasing sodium diformate tended to decrease (quadratic, $P = 0.081$) ADFI and increased (quadratic, $P < 0.001$) G:F. For period 2 (d 32 to 60), there was no evidence for differences ($P > 0.10$) in ADG or ADFI; however, there was a tendency for an

effect (quadratic, $P = 0.093$) on G:F. From d 60 to 93, increasing sodium diformate increased (linear, $P < 0.01$) ADG and ADFI. From d 93 to 117, increasing sodium diformate increased (linear, $P < 0.05$) ADG, ADFI, and G:F. Overall (d 0 to 117), pigs fed increasing sodium diformate had increased (linear, $P < 0.01$) ADG and a tendency for increased (linear, $P = 0.075$) ADFI; however, there was no evidence for differences ($P > 0.10$) in G:F. There were no treatment differences ($P > 0.10$) for any carcass characteristic. In summary, increasing sodium diformate may increase G:F in the early nursey and improve ADG after d 60 (~82 kg) in the finishing period.

INTRODUCTION

Organic acids, such as formic acid, are commonly used as acidifiers in swine diets. Dietary acidifiers have the potential to lower gastrointestinal tract pH, which in turn can improve nutrient digestion, growth performance, and alter gut microbiota of pigs (Suiryanrayna and Ramana, 2015). Within dietary acidifiers, formic acid has one of the lowest acid-binding capacity-4 (ABC-4) values (Lawlor et al., 2005). This high acidification potential indicates formic acid may improve both swine health and growth performance. However, due to the handling characteristics of pure formic acid, it is commonly fed in the form of calcium, sodium, or potassium salts. Sodium diformate is a relatively new salt of formic acid being introduced into the United States market and is the result of combining sodium formate and formic acid into a single, free-flowing product.

The use of formic acid in the form of potassium salts has been extensively studied in both nursery (Tung and Pettigrew, 2006; Poeikhampha and Bunchasak, 2011; Htoo and Molares, 2012) and finishing pigs (Overland, et al., 2000; Mroz et al., 2002). More specifically, potassium diformate has been shown to consistently increase average daily gain (ADG) and gain-to-feed

ratio (G:F; Poeikhampha and Bunchasak, 2011; Htoo and Molares, 2012), benefit intestinal morphology (Poeikhampha and Bunchasak, 2011), and exhibit antimicrobial properties (Canibe et al., 2001) when fed to nursery pigs. Potassium diformate also has been shown to improve both growth performance and carcass characteristics when fed to finishing pigs (Overland et al., 2000). Despite the positive results reported when utilizing potassium diformate in swine diets, there is currently limited research evaluating the use of other formic acid salts, such as sodium diformate.

There are a wide variety of acidifiers on the market, each with different compositions and acidification properties. Therefore, it is important to validate the effects of each new acidifier on pig performance before implementation into commercial diets. Due to the positive effects reported with other formic acid salts, sodium diformate has the potential to act as another effective acidifier in swine diets. However, there is currently limited information evaluating the use of sodium diformate in swine diets. Recently, a study reported improvements in growth performance when sodium diformate was included at 1.2% in nursery diets (Hutchens et al., 2021). Therefore, further research is needed to validate these nursery results as well as provide data on the effects of sodium diformate in finishing pig diets.

MATERIALS AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments.

Experiment 1

The study was conducted at the Kansas State University Segregated Early Weaning Facility located in Manhattan, KS where pigs were housed in two identical, environmentally controlled barns. Pens (1.2 × 1.2 m) had metal tri-bar floors and housed 5 pigs per pen allowing

approximately 0.30 m^2/pig. Each pen contained a cup waterer and a 4-hole, dry self-feeder which provided *ad libitum* access to feed and water.

A total of 360 weanling barrows (DNA 200 × 400, initially 5.9 ± 0.06 kg) were used in a 38-d study. Pigs were randomly assigned to pens and pens were allotted to 1 of 6 dietary treatments with 12 pens per treatment. Diets were fed in 3 phases: phase 1 from weaning to d 9, phase 2 from d 9 to 24, and phase 3 from d 24 to 38. Dietary treatments were formulated to provided none, 0.40, 0.60, 0.80, 1.00, and 1.20% sodium diformate (Formi NDF, ADDCON Nordic AS, Porsgrunn, Norway) added at the expense of corn (Table 3.1). For phase 1 and 2, single base diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center, Manhattan, KS. The base diet was then used to manufacture the 6 treatment diets through additions of sodium diformate at the expense of corn. For phase 3, complete diets were manufactured with a total of 2 batches per treatment. All diets were fed in meal form.

Pig weights and feed disappearance were measured on d 0, 9, 18, 24, 31, and 38 to determine ADG, average daily feed intake (ADFI), and G:F. Feces were collected on d 9 and 24 from 3 pigs per pen via rectal massage to determine fecal dry matter (DM). Fecal samples were dried at 55°C for 48 h and loss of weight was used to determine percentage DM.

Experiment 2

Two rooms at a commercial research site located in south-central Minnesota (Holden Farms, Inc., Northfield, MN) were used for this experiment. Barns had completely slatted, concrete flooring and contained pens (3 × 5.5 m) equipped with a three-hole feeder (Thorp Equipment, Inc., Thorp, WI) and double-sided pan waterer. Pigs were provided *ad libitum* access to feed and water. A computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN) provided daily feed additions.

A total of 2,200 pigs [Duroc sire (PIC 800 or DNA 600) × PIC Camborough; initially 24.2 ± 0.30 kg] were used to conduct a 117-d growth trial. Pens of pigs (25 pigs per pen) were randomly assigned to 1 of 4 dietary treatments in a randomized complete block design with 22 pens per treatment. Dietary treatments were corn-soybean meal-based with the addition of none, 0.25, 0.50, or 0.75% sodium diformate. All diets were manufactured at Bixby Feed Mill, Inc. (Blooming Prairie, MN) and fed in meal form. Diets were fed in 6 phases from 24 to 34, 34 to 66, 66 to 88, 88 to 111, 111 to 120, and 120 to 141 kg. Nutrients for all treatment diets were formulated to meet or exceed the NRC (2012) requirement estimates for finishing pigs in each appropriate weight range (Table 3.2).

Pens of pigs were weighed every two weeks and feed disappearance was measured to determine ADG, ADFI, and G:F. Two weeks prior to the end of the experiment, 4 pigs per pen were weighed and marketed. These pigs were used to determine growth performance but were not included in carcass collection. The remaining pigs were weighed and marketed at the completion of the study. Pigs were transported to a U.S. Department of Agriculture-inspected packing plant (Tyson Fresh Meats, Waterloo, IA). Hot carcass weight (HCW), loin depth, and backfat measurements were collected. Carcass yield was determined using the pen average HCW divided by the pen average final live weight. A proprietary equation from the packing plant was used to calculate the percentage lean.

Statistical analysis

Data from both experiments were analyzed using the GLIMMIX procedure of SAS (v. 9.4, SAS Institute, Inc., Cary, NC). Experiment 1 was analyzed as a completely randomized design with pen as the experimental unit, barn as a random effect, and treatment as the fixed effect. Experiment 2 was analyzed as a randomized complete block design with pen serving as

the experimental unit, treatment serving as the fixed effect, and initial body weight serving as a blocking factor. For both experiments, linear and quadratic contrasts were used to test for the main effects of increasing sodium diformate. Contrast statements were used to test for the main effects of treatment, day, and interaction between treatment and day on fecal DM. For carcass characteristics, individual carcasses served as the observational unit, pen served as the experimental unit, initial body weight served as the blocking factor, and pen was included as a random effect to account for subsampling. Additionally, contrasts were used to analyze carcass characteristics including backfat, loin depth, and percentage lean with HCW weight serving as a covariate. All results were considered significant with $P \leq 0.05$ and marginally significant with $P \leq 0.10$.

RESULTS

Experiment 1

From d 0 to 24 (phase 1 and 2), increasing sodium diformate increased (linear, $P = 0.001$) G:F (Table 3.3). However, sodium diformate did not affect ($P > 0.10$) ADG or ADFI. From d 24 to 38 (phase 3) and the overall study, there was no evidence of differences ($P > 0.10$) for any of the growth performance criteria. Sodium diformate did not affect ($P > 0.10$) body weight (BW) at any timepoint in the trial. Overall mortality was 0.8% and was not influenced ($P > 0.10$) by treatment.

There was no evidence ($P = 0.749$) of an interaction between treatment and day for fecal DM. There was no evidence for differences ($P > 0.10$) in fecal DM on d 9. However, fecal DM decreased (linear, $P = 0.028$; quadratic, $P = 0.097$) as sodium diformate increased on d 24. Additionally, there was evidence for a main effect of day ($P < 0.001$) with fecal DM being lower on d 24 compared to d 9.

Experiment 2

For period 1 (d 0 to 32), increasing sodium diformate tended to decrease (quadratic, $P = 0.081$) ADFI and increased (quadratic, $P < 0.001$) G:F with the greatest G:F observed at 0.25% sodium diformate (Table 3.4). For period 2 (d 32 to 60), there was no evidence of differences ($P > 0.10$) in ADG or ADFI; however, there was a tendency for an effect (quadratic, $P = 0.093$) on G:F, with 0.25 and 0.50% sodium diformate having the greatest G:F. From d 60 to 93, increasing sodium diformate increased (linear, $P < 0.01$) ADG and ADFI. Additionally, from d 93 to 117, increasing sodium diformate increased (linear, $P < 0.05$) ADG, ADFI, and G:F. Overall (d 0 to 117), pigs fed increasing sodium diformate had increased (linear, $P < 0.01$) ADG and a tendency for increased (linear, $P = 0.075$) ADFI; however, there was no evidence for differences ($P > 0.10$) in G:F. Furthermore, increasing sodium diformate increased (linear, $P = 0.005$) final BW on d 117.

There was no evidence of differences ($P > 0.10$) observed for HCW, carcass yield, backfat, loin depth, or percentage lean due to increasing sodium diformate. Overall removals and mortalities were 1.3 and 1.1%, respectively, and were not influenced ($P > 0.10$) by treatment.

DISCUSSION

As the swine industry continues to decrease the usage of antibiotic growth promoters and pharmacological levels of Zn, multiple feed additives including acidifiers, probiotics, prebiotics, and direct-fed microbials are being studied in order to help maintain pig health and performance (Overland, 2001; Liu et al., 2018). Of the various feed additives, acidifiers have been observed to elicit a positive response on growth performance (Kil et al., 2011; Lückstädt and Mellor, 2011; Rao et al., 2023). An extensive review by Kil et al. (2011) evaluating the use of acidifiers in weanling pig diets found that acidifiers elicit a positive response in growth performance when

included during the nursery period; however, there is a high level of variation in response. Furthermore, a review by Rao et al. (2023) focused on the effects of different feed additives on finishing pig growth performance concluded that acidifiers had the potential to positively impact feed efficiency when compared to other feed additives. However, in the feed additive review, implementing acidifiers in finishing diets had a wide range in response with an impact on ADG ranging from -14.9 to 11.4% and an impact on feed efficiency ranging from -9.7 to 11.3% (Rao et al., 2023). Current research is needed to focus on identifying acidifiers that elicit a positive response in swine diets and further understanding their use in different stages of production.

Formic acid is an acidifier characterized as a simple carboxylic acid. Formic acid has gained attention for use within the swine industry due to its high acidification potential. In a study by Lawlor et al. (2005), formic acid had the lowest ABC-4 value of 10 acids. However, due to the corrosiveness and handling characteristics of formic acid, it is often fed in salt form. Forms of formic acid can include calcium, sodium, or potassium salts. There is currently limited research evaluating the use of multiple formic acid salts, including sodium diformate. The sodium diformate product used in this series of trials was a combination of 57% sodium formate and 38.5% formic acid.

Although acidifiers have shown to be variable in response, they are commonly thought to have the most consistent effects on growth performance when utilized during the nursery period. Weaning is a crucial period of a pig's life where they are challenged with a multitude of factors including changes in environment, diet, and physiological development. Specifically, due to the substantial change in diet, newly weaned pigs exhibit reduced stomach acid secretions (Pluske, 2016). An addition of an acidifier may result in improvements in growth performance due to a decrease in gastric pH. Kil et al. (2011) reported that throughout 17 studies, 76% of the treatment

groups supplemented with a dietary acidifier experienced reductions in stomach pH. This reduction in stomach pH may lead to impacts on gut microbiota and nutrient digestion. This has been validated utilizing potassium diformate which has shown to consistently improve ADG and feed efficiency (Poeikhampha and Bunchasak, 2011; Htoo and Molares, 2012), benefit intestinal morphology (Poeikhampha and Bunchasak, 2011), and exhibit antimicrobial properties (Canibe et al., 2001) when fed to nursery pigs. The previous research utilizing potassium diformate aligns with the improvement in feed efficiency reported during the first two nursery phases in experiment 1. However, in the present study, the improvements in feed efficiency did not continue into phase 3 or translate into differences for the overall experimental period. This contradicts a previous study (Htoo and Molares; 2012) utilizing potassium formates that observed no differences in the early nursery period (d 0 to 14), but found improvements in ADG and G:F in both the late nursery (d 15 to 35) and overall period (d 0 to 35). The ADG and feed intake for the first 14 d of the Htoo and Molares (2012) study was notably lower than in the present study. The authors theorized that this lower feed intake during the prestarter phase may have been the cause for the lack of differences at the beginning of the study, with improvements in performance seen later in the study when pigs began to consume more feed. Therefore, the response in acidifiers within the nursery period may be driven by the health and performance level of those pigs when entering the nursery or by the differences in effectiveness of the acidifier itself.

Hutchens et al. (2021) evaluated the use of 1.2% sodium diformate fed in the first two phases of the nursery (d 0 to 21). Pigs fed 1.2% sodium diformate had increased ADG and G:F. This improvement in G:F observed during the early nursery phase when using 1.2% sodium diformate aligns with the study herein, with the study by Hutchens et al. (2021) reporting a 7%

improvement in G:F and the present study finding a 4% improvement in G:F. The similarity in results were expected as both trials were conducted utilizing similar research conditions, genetics, and the same sodium diformate product. However, Hutchens et al. (2021) found a tendency for an increased ADG and d 42 BW in the overall period despite only feeding sodium diformate for the first 2 phases. This research indicates sodium diformate can consistently impact growth performance of pigs during the early nursery period, but further data is needed to understand how to translate those differences into the later nursery phase.

In addition to improvements in growth performance, acidifiers may have the potential to reduce the incidence of diarrhea in nursery pigs. Specifically, recent work evaluating different ABC-4 values of weanling pig diets have reported an increase in fecal DM as acid-binding capacity is decreased (Stas et al., 2023). However, studies evaluating either fecal score, incidence of diarrhea, or fecal DM have observed no differences with the use of dietary acidifiers alone (Htoo and Molares, 2012; Grecco et al., 2018; Hutchens et al., 2021). In experiment 1, feeding sodium diformate did not impact fecal DM on d 9, but decreased fecal DM on d 24. This response was unexpected as previous research has shown either a positive or no response of diet acidification on fecal DM. Fecal DM can be affected by multiple factors including feed intake, water consumption, and disease status. In experiment 1, pigs were healthy and had an adequate feed intake; therefore, fecal DM was relatively high with ranges from 24.8 to 26.9% on d 9 and 21.9 to 25.7% on d 24. Although there was a decrease in fecal DM on d 24, it was still in a healthy range for a nursery pig of that age. Ultimately, further research would be needed to investigate the response of sodium diformate on fecal DM during a health challenge to elicit a better understanding of its impact on the gastrointestinal tract.

Despite the previous literature outlining the positive benefits of formic acid salts on nursery pig performance, there has been little literature investigating its effects in finishing pig diets. Overland et al. (2000) evaluated the use of calcium formate, sodium formate, and potassium diformate fed to finishing pigs. Overall, potassium diformate increased ADG during the experimental period; however, both calcium formate and sodium formate did not have an impact on growth performance. In experiment 2, there were improvements in both ADG and ADFI which are similar to the those outlined by Overland et al. (2000) when utilizing potassium diformate. The interesting comparison between these trials is the benefits reported when utilizing sodium diformate, but lack of response when using sodium formate. This indicates the importance of validating each acidifier as they enter the market, even if they are thought to be similar. Most of the response to sodium diformate during the present study was found in the late finishing period (after d 60), while the improvements in ADG utilizing potassium diformate were largely found in the early grower period. Similar to the studies presented utilizing acidifiers in the nursery period, there seems to be an inconsistency in magnitude and timing of the benefits reported when utilizing acidifiers in the finishing period.

Although the majority of benefits in growth response from sodium diforamte were seen in late finishing, there was an improvement in G:F up to 0.50% sodium diformate during the first 32 d of the study. Interestingly, this aligns with the early improvements in G:F seen in the nursery trial when including increasing levels of sodium diformate. Tung and Pettigrew (2006) identified acidifiers as being a useful tool to help pigs overcome periods of high stress and disease challenge. Therefore, the improvements in G:F may be driven by the initial introduction of an acidifier to the diet or the inclusion of an acidifier during a period of stress such as at weaning or transition to the finisher. It is important to note that there was a high health status and

low levels of removals and mortalities throughout both studies. Therefore, additional research is needed to understand if sodium diformate could elicit a greater response under more challenging conditions.

In summary, these data suggest increasing sodium diformate has the potential to increase G:F in the early nursey period but did not affect performance in the late nursery. This indicates that the acidification potential of sodium diformate has the greatest benefit immediately post-weaning, when the pig is the most challenged. However, the benefits post-weaning did not translate into improved performance for the overall nursery period. Furthermore, feeding increasing sodium diformate increased ADG and ADFI after d 60 (~82 kg) in the finishing period but carcass traits were unaffected. In both studies, improvements were seen to the highest inclusion level, 1.20% and 0.75%, respectively. Additional research is needed on target feeding sodium diformate during specific stages of the nursery and finishing period to maximize performance while being economical for producers.

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Table 3.1. Diet composition (as-fed basis), Exp.1[1]

Item, %	Phase 1	Phase 2	Phase 3
Ingredients, %			
Corn	44.85	50.12	64.18
Soybean meal	17.41	23.76	31.78
Spray-dried whey	10.00	---	---
Whey permeate	10.00	10.00	---
Dried distillers grains with solubles	5.00	7.50	---
Fish meal	2.50	---	---
Fermented soybean meal[2]	4.00	3.85	---
Spray-dried bovine plasma	2.00	---	---
Soybean oil	1.00	1.00	---
Monocalcium P	0.80	1.00	1.00
Calcium carbonate	0.41	0.40	0.88
Zinc oxide	0.40	0.25	---
Sodium chloride	0.30	0.50	0.60
Vitamin premix[3]	0.25	0.25	0.25
Trace mineral premix[4]	0.15	0.15	0.15
Phytase[5]	0.08	0.08	0.08
L-Lys-HCL	0.40	0.55	0.50
DL-Met	0.19	0.23	0.21
L-Thr	0.17	0.22	0.21
L-Trp	0.02	0.04	0.04
L-Val	0.08	0.12	0.13
Sodium diformate[6]	+/-	+/-	+/-
Total	100	100	100
Calculated analysis			
Standardized ileal digestible (SID) amino acids, %			
Lys, %	1.35	1.35	1.35
Ile:Lys	57	57	56
Leu:Lys	120	118	115
Met:Lys	36	38	36
Met and Cys:Lys	56	56	58
Thr:Lys	64	63	63
Trp:Lys	19.2	19.2	19.3
Val:Lys	70	70	70
Total Lys, %	1.51	1.51	1.50
NE, kcal/kg	2,544	2,504	2,418
SID Lys:NE, g/Mcal	5.30	5.39	5.58
Crude protein, %	21.5	21.5	21.3
Ca, %	0.66	0.54	0.71
P, %	0.66	0.61	0.61
STTD P, %[7]	0.59	0.51	0.48

[1]Phase 1 diets were fed from 5.9 kg to 7.1 kg, phase 2 diets were fed from 7.1 kg to 13.4 kg, and phase 3 diets were fed from 13.4 kg to 21.7 kg.

[2]MEPro, Prairie Aquatech, Brookings, SD.

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

[4]Provided per kg of diet: 110 mg Zn from zinc sulfate; 110 mg Fe from iron sulfate; 33 mg Mn from manganese oxide; 17 mg Cu from copper sulfate; 0.30 mg I from calcium iodate; 0.30 mg Se from sodium selenite.

[5]Ronozyme HiPhos 2700 GT, DSM Nutritional Products, Parsippany, NJ was included at 2,000 FTU/kg to provide an estimated release of 0.12% STTD P for all diets.

[6]Sodium diformate (Formi NDF, ADDCON Nordic AS, Porsgrunn, Norway) was included at none, 0.40, 0.60, 0.80, 1.00, and 1.20% at the expense of corn.

[7]STTD P = Standardized total tract digestible phosphorus.

Table 3.2. Composition of experimental diets (as-fed basis), Exp. 2[1]

Item	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5	Phase 6
Ingredients, %						
Corn	42.96	52.96	60.73	64.54	69.38	82.73
Soybean meal	24.50	14.50	6.75	3.00	3.25	15.50
Dried distillers grains with solubles	30.00	30.00	30.00	30.00	25.00	---
Monocalcium P	0.05	---	---	---	---	0.05
Calcium carbonate	1.20	1.10	1.10	1.10	1.10	0.80
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50
L-Lys-HCl	0.36	0.49	0.51	0.49	0.45	0.19
Thr[2]	0.06	0.12	0.12	0.11	0.11	0.07
L-Trp	0.00	0.03	0.05	0.05	0.04	0.00
Vitamin premix with phytase[3]	0.20	0.15	0.13	0.13	0.10	0.10
Trace mineral premix	0.15	0.13	0.10	0.10	0.08	0.08
Copper sulfate	0.03	0.03	0.03	---	---	---
Sodium diformate[4]	+/-	+/-	+/-	+/-	+/-	+/-
Total	100	100	100	100	100	100
Calculated analysis						
Standardized ileal digestible (SID) amino acids, %						
Lys, %	1.15	1.02	0.86	0.75	0.72	0.72
Ile:Lys	68	61	58	58	58	66
Leu:Lys	167	166	177	191	189	163
Met:Lys	30	29	31	33	33	30
Met and Cys:Lys	57	56	59	63	63	60
Thr:Lys	63	63	63	64	65	65
Trp:Lys	18	18	18	18	18	18
Val:Lys	78	73	72	75	74	76
His:Lys	46	43	43	44	44	47
Total Lys, %	1.34	1.18	0.99	0.88	0.83	0.79
NE, kcal/kg	2,465	2,489	2,504	2,513	2,533	2,604
SID Lys:NE, g/Mcal	4.67	4.11	3.42	3.00	2.83	2.76
Crude protein, %	23.2	19.5	16.4	15.0	14.0	13.2
Ca, %	0.64	0.58	0.53	0.51	0.49	0.43
STTD P, %[5]	0.41	0.38	0.35	0.34	0.31	0.26

[1]Phases were fed from approximately 24 to 34, 34 to 66, 66 to 88, 88 to 111, 111 to 120, and 120 to 141 kg body weight, respectively.

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

[3]Phytase was included at 880 FTU/kg in phase 1 (estimated 0.14% STTD P release), 660 FTU/kg in phase 2 (0.14% STTD P release), 550 FTU/kg in phases 3 and 4 (0.13% STTD P release), and 440 FTU/kg in phases 5 and 6 (0.12% STTD P release).

[4]Formi NDF (ADDCON Nordic AS, Porsgrunn, Norway) at none, 0.25, 0.50, and 0.75% of the diet was included at the expense of corn.

[5]STTD P = Standardized total tract digestible phosphorus.

Table 3.3. Effects of increasing sodium diformate on nursery pig performance and fecal dry matter, Exp. 1[1]

	Sodium diformate, %[2]							*P* =	
Item	0	0.40	0.60	0.80	1.00	1.20	SEM	Linear	Quadratic
BW, kg									
d 0	5.9	5.9	5.9	5.9	5.9	5.9	0.06	0.905	0.911
d 24	13.3	13.2	13.4	13.6	13.4	13.3	0.20	0.671	0.630
d 38	21.7	21.6	21.8	21.8	21.6	21.7	0.33	0.980	0.956
d 0 to 24 (Phase 1 and 2)									
ADG, g	306	302	312	320	313	304	8.5	0.683	0.420
ADFI, g	393	386	396	396	391	374	9.2	0.356	0.237
G:F, g/kg	779	783	788	807	799	812	8.2	0.001	0.629
d 24 to 38 (Phase 3)									
ADG, g	600	599	602	583	585	597	15.8	0.511	0.803
ADFI, g	852	867	858	849	848	856	17.0	0.822	0.792
G:F, g/kg	705	692	702	686	689	697	9.7	0.350	0.421
d 0 to 38 (Overall)									
ADG, g	414	412	419	417	413	410	8.8	0.822	0.626
ADFI, g	561	563	566	563	560	548	11.0	0.443	0.332
G:F, g/kg	738	731	740	740	738	748	5.6	0.186	0.293
Fecal DM, %[3]									
d 9	26.26	26.77	26.85	26.91	24.80	25.41	1.146	0.299	0.287
d 24	24.21	25.70	25.32	23.80	21.92	23.41	0.914	0.028	0.097

[1]A total of 360 weanling barrows (DNA 200 × 400, DNA; initially 5.9 ± 0.06 kg) approximately 21 days of age were used in a 38-d experiment with 5 pigs per pen and 12 pens per treatment.

[2]Formi NDF, ADDCON Nordic AS, Porsgrunn, Norway.

[3]Feces from three piglets from each pen were pooled, weighed, and dried to measure fecal dry matter. Treatment × day, $P = 0.749$; Treatment, $P = 0.120$; Day, $P < 0.001$.

Table 3.4. Effects of increasing sodium diformate on growth performance and carcass characteristics of finishing pigs, Exp. 2[1]

	Sodium diformate, %[2]					*P* =	
Item	0	0.25	0.50	0.75	SEM	Linear	Quadratic
BW, kg							
d 0	24.2	24.2	24.3	24.2	0.30	0.808	0.538
d 32	53.4	53.5	53.2	53.3	0.40	0.445	0.834
d 60	81.8	82.2	81.9	81.9	0.47	0.954	0.359
d 93	116.3	117.1	117.0	117.1	0.48	0.118	0.338
d 117	138.8	139.8	140.2	140.4	0.70	0.005	0.307
Period 1 (d 0 to 32)							
ADG, kg	0.91	0.91	0.90	0.90	0.005	0.287	0.641
ADFI, kg	1.72	1.70	1.70	1.71	0.014	0.424	0.081
G:F, g/kg	528	537	533	528	2.5	0.735	< 0.001
Period 2 (d 32 to 60)							
ADG, kg	1.01	1.03	1.03	1.02	0.008	0.646	0.166
ADFI, kg	2.46	2.48	2.47	2.47	0.018	0.810	0.580
G:F, g/kg	412	415	416	413	1.9	0.625	0.093
Period 3 (d 60 to 93)							
ADG, kg	1.04	1.05	1.06	1.07	0.006	0.004	0.970
ADFI, kg	3.01	3.02	3.05	3.06	0.016	0.008	0.817
G:F, g/kg	348	347	348	349	2.0	0.462	0.797
Period 4 (d 93 to 117)							
ADG, kg	1.07	1.09	1.10	1.12	0.015	0.003	0.708
ADFI, kg	3.59	3.63	3.63	3.66	0.022	0.017	0.798
G:F, g/kg	299	301	304	305	3.5	0.035	0.782
Overall (d 0 to 117)							
ADG, kg	1.00	1.01	1.01	1.02	0.004	0.004	0.236
ADFI, kg	2.60	2.61	2.62	2.63	0.014	0.075	0.905
G:F g/kg	385	387	388	387	1.9	0.313	0.212
Carcass characteristics							
HCW, kg	101.7	102.4	103.1	102.4	0.60	0.168	0.107
Carcass yield, %	73.4	73.3	73.4	72.9	0.22	0.117	0.334
Backfat, mm[3]	13.2	13.1	12.9	13.0	0.15	0.207	0.380
Loin depth, mm[3]	73.0	73.2	72.7	72.5	0.46	0.220	0.545
Lean, %[3]	57.3	57.3	57.3	57.2	0.08	0.719	0.383
Removals, %	1.82	0.91	0.91	1.46	0.570	0.666	0.144
Mortality, %	1.09	0.91	1.09	1.46	0.511	0.545	0.562
Mortality and removals, %	2.91	1.82	2.00	2.91	0.717	0.934	0.133

[1]A total of 2,200 pigs (initially 24.2 ± 0.30 kg) were used in a 117-d growth trial with 25 pigs per pen and 22 replicates per treatment.

[2] Formi NDF, ADDCON Nordic AS, Porsgrunn, Norway.

[3]Adjusted using HCW as covariate.

Chapter 4 - A review of soybean processing by-products and their use in swine and poultry diets

ABSTRACT

Due to its importance in animal feed, soybean meal has been extensively studied to optimize its use in livestock diets. Despite extensive research, the industry has not fully characterized specific areas of soybean processing such as the inclusion of soybean by-products added back to soybean meal during processing. Soybean processing by-products can encompass a large variety of materials including weeds and foreign material, soybean hulls, gums, soapstocks, lecithins, spent bleaching clays, and deodorizer distillates. Despite the potential for being added back to soybean meal when a crushing plant is integrated with an oil refinery, there is currently limited information on the composition of many of these soybean processing by-products and their subsequent effects on soybean meal quality and animal performance. Therefore, there may be opportunities for a new area of research focused on soybean processing by-products and their optimal use within the livestock feed industry. This review summarizes the current information on soybean by-products with a focus on identifying the areas with the greatest potential for future research in swine and poultry nutrition.

INTRODUCTION

With high crude protein (44 to 48%) and energy (swine, net energy: 2,233 kcal/kg; poultry, apparent metabolizable energy: 2,473 kcal/kg), soybean meal serves as an affordable, high-quality protein source in animal feeds (Nahashon and Kilonzo-Nthenge, 2011; Lee et al., 2022; Stein, 2023). According to the United Soybean Board, in 2021, 78% of the total soybean meal produced was fed to poultry and swine (equivalent to 26.8 million metric tons). Initially

identified by Hayward in 1970, and re-emphasized by Ruiz et al. in 2020, there are three areas of soybean meal research that continue to dominate the industry: amino acid digestibility, evaluation of anti-nutritional factors, and energy valuation. Despite extensive research, the industry has not fully characterized other specific areas of soybean processing such as the inclusion of soybean by-products added back to soybean meal during processing.

Soybean processing by-products can encompass a large variety of materials including weeds and foreign material, soybean hulls, gums, soapstocks, lecithins, spent bleaching clays, and deodorizer distillates (Table 4.1). Once produced, by-products may be disposed of as waste, sold to an identified market to add value, or added back to soybean meal. Despite the potential for being added back to soybean meal when a crushing plant is integrated with an oil refinery, there is currently limited information on the composition of many of these soybean processing by-products and their subsequent effects on soybean meal quality and animal performance (Overland et al., 1993a,b; Woerfel, 1995a,b; Bruce et al., 2006). Therefore, there may be opportunities for a new area of research focused on soybean processing by-products and their optimal use within the livestock feed industry. This need for understanding the optimal utilization of soybean by-products will only increase as the need for soybean oil continues to grow to meet the demand of renewable fuels. This review aims to summarize the current information on soybean by-products (weeds and foreign material, hulls, gum, soapstocks, lecithins, bleaching clays, and deodorizer distillates) with a focus on identifying the areas with the greatest potential for future research in swine and poultry nutrition.

BACKGROUND ON SOYBEAN PROCESSING

The soybean oil milling industry developed out of the demand for soybean oil both for human consumption and industrial application (Shurtleff and Aoyagi, 2004). This demand is

expected to grow as the production of sustainable biofuels is rapidly expanding (Wightman, 2021). This increase in demand will not only increase the production of refined soybean oil, but also the by-products produced throughout the oil extraction process. The main outputs of soybean processing are crude soybean oil and soybean meal. Crude oil is not viable for use in many applications, such as human consumption. Therefore, a sequence of further processing steps must be completed to refine the oil. Consequently, multiple additional by-products are produced which must find a market or appropriate disposal method.

Efficient soybean processing begins with the quality of incoming soybeans. Despite the importance soybean harvesting, handling, and storage have on the quality of the resulting products, soybean processors have minimal control over these initial factors (Erickson, 1995a). Understanding variability of incoming soybeans allows processors to adjust processing steps appropriately. Even small changes in soybean oil or soybean meal production can have large impacts on soybean processing by-products. For example, if incoming soybean quality is lower, resulting in soybean meal close to the minimum protein target, there is very little opportunity for processors to add by-products back to soybean meal. Adding by-products devoid of protein to soybean meal would dilute the percentage of protein in that batch potentially leading to missed specifications and increased risk of claims. Therefore, this presents an immediate challenge when aiming to characterize the composition of by-products and their use in animal diets as their presence in soybean meal is inconsistent throughout the industry and can be impacted by multiple factors.

Following arrival at the soybean processing facility, soybeans will go through a series of steps in preparation for oil extraction which may include cleaning, drying, cracking, dehulling, conditioning, and flaking (Woerfel, 1995b; Figure 4.1). Cleaning and dehulling soybeans are

essential to maximize the protein content in soybean meal and increase extractor throughput for soybean processors (Woerfel, 1995b). Extractor throughput, which serves as a crucial economical factor for processors, is the rate at which a plant can run soybeans through the extractor in order to capture the oil. Once separated from the soybeans, hulls and other screenings such as weeds and foreign material become a by-product stream for soybean processors to manage, further process, and market for other applications. Although weeds, foreign materials, or hulls are not commonly added back to soybean meal in modern production, the quantity of these residual products remaining in the soybean meal may vary. Therefore, cleaning and preparation of incoming soybeans using screens and aspiration have the potential to impact soybean meal quality. These screenings are frequently either added to the removed soybean hulls or disposed of as waste.

Following cleaning and dehulling, soybeans are then cracked into smaller particles and prepared for further processing. These small soybean pieces will then be flaked facilitating the rupture of the oil cells. Then the flakes are extracted using hexane (Lamsal et al., 2007; Johnson, 2008). Due to extraction efficiency and preparation of soybeans, it is now possible to produce soybean meal with as little as 0.8% residual oil (Johnson, 2008; NOPA, 2021). Once the oil is extracted, it will continue to be further processed resulting in refined soybean oil and several by-products.

Oil processing

The liquid portion leaving the extractor is commonly termed miscella and is a combination of approximately 25% oil and 75% solvent. This mixture enters a series of evaporators and strippers to maximize the recovery of high-quality crude oil, while minimizing residual hexane (Demarco and Gibon, 2020). Depending on the desired product, crude oil can go

through multiple refining steps including degumming, neutralization, bleaching, and deodorization (Erickson, 1995a).

Degumming is commonly the first step in oil refining and involves removal of phosphatides from the oil. Depending on the specific plant and quality of incoming oil, degumming is not always implemented. There are multiple degumming processes that may be used including water, acid, dry, or enzymatic degumming (Dijkstra, 2010). Conventional degumming utilizes water to hydrate phosphatides that can then be centrifuged from the oil. Newer methods of degumming use enzymes, called phospholipases, to hydrolyze phospholipids (Yang et al., 2008). Although enzymatic degumming is thought to be a gentler method with a lower oil loss, conventional methods of degumming are still most used. This process ultimately yields degummed oil and the by-product known as soybean gums (Erickson, 1995b; NOPA, 2021).

The next step of oil refining is neutralization and can be implemented on either crude or degummed oil. Soybean processors often use neutralization through caustic refining to remove remaining phosphatides and neutralize free fatty acids (Erickson, 1995c). Caustic refining is defined as the treatment of an oil with an aqueous alkali solution, typically utilizing sodium hydroxide, also known as lye. Sodium hydroxide will react with free fatty acids in the oil creating soaps that can be removed through centrifugation coining the term “soapstocks” (Erickson, 1995c; NOPA, 2021). These soapstocks constitute an additional by-product stream for soybean processors. As expected, a larger quantity of soapstocks will be produced if neutralizing crude oil that has not been through the degumming process, compared to an already partially refined, degummed oil. If plants utilize a physical refining step in lieu of caustic refining, free

fatty acids will be removed through steam injection; therefore, not resulting in the generation of soapstocks (Gharby, 2022).

Following refining, oil can be bleached and deodorized to produce a final, fully refined oil product. Bleaching focuses on reducing oxidation products, removing remaining soaps and phosphatides, and extracting pigments. Acid-activated clays are commonly used during the bleaching process and can absorb pigments and other undesirable compounds (Erikson, 1995d; Abedi et al., 2015). Following bleaching, oils may be deodorized by application of steam. Steam will volatilize any remaining undesirable compounds resulting in a colorless, odorless oil product (Zehnder, 1995). These final oil refining processes result in additional by-products termed "spent bleaching clay" and "deodorizer distillate", respectively (Erickson, 1995d).

Through these processing steps, multiple by-products can be produced including weeds and foreign material, soybean hulls, gums, soapstocks, spent bleaching clay, and deodorizer distillates. Despite previous research and the long history of oil refining, there is limited literature outlining the composition and nutritional value of soybean processing by-products. Therefore, there is a potential that the soybean processing and animal feed industries are overlooking an important component impacting soybean meal quality and the resulting animal feed products.

SOYBEAN BY-PRODUCTS IN NON-RUMINANT DIETS

Weeds and foreign material

Although representing a small portion of soybean meal, weeds and foreign material can have a notable impact on animal growth performance and health. Weeds and foreign material are comprised of foreign materials including plant parts, broken beans, weed seeds, dirt, whole beans, pods, insects, and corn (Lin, 1996). Foreign materials can potentially impact the

nutritional value of soybean meal, even at low inclusion levels, due to their high fiber content and potential for anti-nutritional factors. A study by Bruce et al. (2006) evaluated the impact of adding weeds and foreign material back at 2% of the soybean meal on nutrient digestibility in swine diets. They observed that both apparent ileal digestibility and standardized ileal digestibility of amino acids were lower when weeds and foreign material were added back to soybean meal. This could potentially be due to antinutritional factors including tannins and phenolics residing in the various weeds and seeds (Reed, 1995; Bruce et al., 2006). Additionally, having excess weeds and foreign materials in soybeans will dilute the nutrient contents as well as pose a potential risk to animal health through high levels of toxic contaminates (List et al., 1979). In modern soybean processing, it is not common to add weeds and foreign material back to the soybean meal. Weeds and foreign material screened from the soybeans are typically either added to pelletized soybean hulls or disposed of as waste.

Soybean hulls

Soybean hulls constitute approximately 5% of the total soybean and as a result, are the greatest amount of by-product produced during soybean processing (Ferrer et al., 2016; Liu and Li, 2017). Soybean hulls are comprised of varying amounts of cellulose, hemicellulose, lignin, pectins, and proteins. Therefore, hulls are lignocellulosic material that are high in fiber and contain elevated peroxidases (Liu and Li, 2017). In addition to potential uses in industrial applications (treatment of wastewater and production of filaments), most hulls will end up in animal diets either through being added back to soybean meal or are in the form of loose or pelletized soybean hulls (Woerfel, 1995a).

Due to the low energy and high fiber content of soybean hulls, they are not commonly fed in swine diets (NRC, 2012). Past literature shows a consistent reduction in gain:feed ratio as

nursery pigs are fed increasing levels of soybean hulls (Kornegay, 1978; Kornegay et al., 1995). This response is largely driven by the dilution of energy as more soybean hulls are added to the diet. Finishing pigs have an increased ability to ferment fiber compared to nursery pigs due to a maturation of the gastrointestinal tract and as a result are better equipped to handle additional fiber in the diet (Noblet and Van Milgen, 2004). Bowers et al. (2000) reported finishing pig diets could include up to 3% added soybean hulls without affecting performance. Regardless of this increased ability to handle fiber, high levels of soybean hulls (between 6 to 30%) have resulted in reduced average daily gain (ADG) and feed efficiency in finishing pigs (Kornegay, 1978; Bowers et al., 2000; Stewart et al., 2013) unless maintaining the ME/kg with the addition of fats or oils (Bowers et al., 2000). In addition to diluting the energy content of the diet, soybean hulls may also have a negative impact on digestibility. Dilger et al. (2004) observed that when feeding up to 9% soybean hulls, a 0.2% decrease in ileal digestibility of indispensable AA may occur for every 1% of added soybean hulls. This agrees with data of Kornegay (1978) and Bruce et al. (2006) who observed decreases in energy and protein digestibility when soybean hulls were fed to growing pigs. Despite the diluted energy and potential reductions in digestibility, soybean hulls may have practicality in gestating sow diets, specifically within group housing systems. Fibrous ingredients, such as soybean hulls, have the potential to manage satiety of gestating sows when fed at greater than 100 g of soluble fiber per d, ultimately helping limit aggression towards pen mates (Wensley et al., 2022).

Research evaluating soybean hulls in poultry diets has been inconsistent. Broilers fed high fiber diets often results in a reduction in body weight gain (Jha and Mishra, 2021). However, there have been reported studies finding improvements in performance when soybean hulls are fed at a moderate level (approximately 5%) in poultry diets noting improvements in

gain (Masoudi and Bojarpou, 2020, Tejeda and Kim, 2020). The variation in response to fiber in broiler diets is largely driven by the fiber source and inclusion level. In addition to performance, research has begun to focus on other benefits to feeding fiber sources, such as soybean hulls, in poultry diets. Like swine, one focus is on satiety. High fiber diets have shown a potential to reduce cannibalism in poultry production systems, potentially serving as a feasible replacement for beak trimming in laying hens (Hartini et al., 2002). Furthermore, feeding soybean hulls has consistently shown improvements in organ development, indicated by increased gizzard and intestinal weights, potentially leading to the benefits in growth performance seen when fed at moderate levels to birds (Tejeda and Kim, 2020; Jha and Mishra, 2021). Additionally, other studies have reported beneficial aspects of soybean hulls in swine and poultry diets including stimulating growth of the gastrointestinal tract, reduced digesta passage rate, and improved intestinal health (Pascoal et al., 2015; Jha and Mishra, 2021). Therefore, current research should focus on the health by nutrition interaction associated with soybean hulls. Ultimately, soybean hulls are likely to be pelletized and marketed as a separate by-product stream which can act as a dietary fiber source in monogastric diets but are most commonly used in ruminant feeds.

Soybean gums and soapstocks

Soybeans generate the highest percentage of gums compared to other vegetable oils, making this an important by-product for soy processors (Erickson, 1995b). The composition of gums is influenced by multiple factors including the quality of incoming soybeans, differences in processing procedures, and the age and efficiency of plant machinery. Although it will vary across the industry, the Practical Handbook of Soy Oil Processing and Utilization outlines the general composition of gums as 35% soybean oil, 17% phytogycolipids, 16% phosphatidyl choline, 14% phosphatidyl ethanolamine, 10% phosphatidyl inositol, and 7% carbohydrates

(Erickson, 1995b). In addition to gums, soapstocks are another by-product of the refining process. If left untreated, soapstocks will solidify and ferment leading to storage safety issues (Gerde et al., 2020). After collection of soapstocks, they will either be neutralized or acidulated for animal feed applications. Once the product is acidulated, the composition is approximately 20 to 30% oil, 65 to 70% fatty acids, and 5% sterols and oxidized components (Erickson, 1995c).

Gums and soapstocks are both products commonly added back to soybean meal. It is most economical to include these by-products back into the soybean meal to reduce the cost of transportation or disposal when a crushing facility is integrated with an oil refinery. Both gums and soapstocks will contain free fatty acids and phospholipids, ultimately allowing them to potentially be used as a source of energy in livestock feed (Gerde et al., 2020). One challenge with characterizing the effects of adding soybean gums and soapstocks back to soybean meal is the variation in composition and quantity. Current literature has not outlined the quantity of gums or soapstocks typically added back to soybean meal. This is likely because the quantities that are added back to the soybean meal at the desolventizing, toasting, drying, and cooling (DTDC) step is inconsistent and largely depends on the quality of the initial soybeans processed. Based on the ranges in analyzed nutrient compositions of soybean meal and personal communications throughout the soybean crushing facilities, an estimated inclusion of gums and soapstocks can range from 0 to 2%. The variation in the production of these two by-products has been documented (Erickson, 1995b,c; O'Brien, 2008) due to factors such as initial soybean quality, efficiency of the machinery, differences in the degumming process, and differences in the caustic refining process. However, there is currently no literature quantifying this variation across soybean processing plants.

Although there is currently limited data on composition, studies have aimed to evaluate the use of gums and soapstocks in monogastric diets. Soapstocks have been heavily studied by the poultry industry due to their potential as a low-cost energy source that could also successfully act as a pigment (Menge and Beal, 1973; Vieira et al., 2006; Borsatti et al., 2018). Specifically, poultry studies have indicated that replacing animal and vegetable fats and oils with dried soybean soapstock (Menge and Beal, 1973) or replacing degummed oil with acidulated soybean soapstock (Vieira et al., 2006) resulted in similar performance indicating soybean by-products may act as an adequate energy source in poultry formulations. In addition to serving as a more affordable, but comparable source of energy, feeding soapstocks led to improved shank pigmentation indicating an additional benefit as a pigmenter (Menge and Beal, 1973). Broiler research has outlined a metabolizable energy value for acidulated soapstocks which range from approximately 6,700 to 7,500 kcal apparent metabolizable energy corrected for N/kg depending on the mixture of the product sourced and age of the bird (Gaiotto et al., 2000; Freitas et al., 2005; Borsatti et al., 2018).

The swine industry has also investigated the impact of soybean gums and soapstocks on growth performance and digestibility. Bruce et al. (2006) characterized the effects of soybean gums and soapstocks when added back during processing on the digestibility of soybean meal in swine. They aimed to utilize quantities of gums and soapstocks representative of those typically added back to soybean meal. This included evaluating both 1 and 3% added gums to soybean meal as well as 0.50 and 1.5% added soapstocks to soybean meal. They observed that increasing levels of gums and soapstocks added to soybean meal decreased the apparent ileal and standardized ileal digestibility of crude protein and amino acids (Arg, His, Leu, Lys, Phe, Val, Ala, and Gly). Additionally, they tested a combination of 3% gums, 1.5% soapstocks, and 2%

weeds and foreign material added to soybean meal resulting in 6.5% total by-product add-backs. This combination consistently produced the lowest apparent and standardized ileal amino acid digestibility when compared to individual by-product treatments. Inversely, soybean by-products did not appear to have an effect on apparent total tract digestibility of protein, fat, calcium, magnesium, or phosphorus when feeding soybean soapstock up to 10% of the diet (Atteh and Leeson, 1985) or apparent total tract digestibility of dry matter, crude protein, ether extract, and gross energy when feeding olive oil soapstock at 7.5% of the basal diet (Rojas-Cano et al., 2014). The potential decrease in digestibility of amino acids may be a result of chelating amino acids in the small intestine making them unavailable (Woerfel, 1981a). Additional research is needed to understand both the risks and benefits associated with added gums and soapstocks on nutrient digestibility in modern poultry and swine diets utilizing soybean meal reflective of today's manufacturing processes.

Gums and soapstocks contain residual oil from the refining process and thus may serve as a potential energy source for swine and poultry. A study using up to 8% olive oil soapstock observed no significant effect on ADG or feed efficiency of growing pigs (47 kg). When olive oil soapstock was fed to finishing pigs (57 kg), both an increase in ADG and improvement in feed efficiency were reported (Rojas-Cano et al., 2014). Lazor and Biocanin (1970) observed an improvement in ADG and feed efficiency when pigs were fed diets with 2% added sunflower soapstocks. The increase in performance seen in these studies was largely driven by the increased energy density of the diet. In contrast, Atteh and Leeson (1985) did not find significant differences in feed efficiency when feeding finishing pigs 5% soapstocks, but ADG decreased with the addition of 10% soapstocks. These diets were formulated to be isocaloric and contained similar levels of soybean meal as a protein source. Therefore, the cause of this decrease in

performance is unknown and may indicate a limit for which soybean by-products can be included in swine diets. Further research is needed to characterize the composition, variation, and nutritional value of soybean gums and soapstocks before they can be utilized in monogastric diets.

Lecithin

Lecithin is derived from further processing soybean gums. If soybean processors are producing lecithins, the soybean gums produced during degumming will then be de-oiled and will go through alcohol fractionation (Gerde et al., 2020). The most difficult step in lecithin production is drying. The gums must go from approximately 50% to less than 1% moisture through a highly controlled heating process.

Lecithins can act as an emulsifier and antioxidant which has increased its demand in baking and food, cosmetic, and industrial applications (Erickson, 1995b). Lecithin may also be used in animal feeds. The addition of lecithins has the potential to interact with added fats which is thought to improve fat digestion. However, multiple studies have failed to find a positive response in both digestibility and growth performance when lecithins were added to swine diets (Overland et al., 1993a,b; Soares and Lopez-Bote, 2002; Gatlin et al., 2005; Kerr and Shurson, 2017). A study feeding lysolecithin found improved digestibility of multiple fat sources; however, this response did not translate to improved growth performance (Jones et al., 1992). Although the emulsification benefits do not result in improved growth performance in swine, lecithins have shown benefits within the poultry industry. A review by Ullah et al. (2023) reported multiple benefits of lecithins in poultry diets including improved production, nutrient digestibility, and health status. Furthermore, they observed that as little as 2% added lecithin in poultry diets can lead to improved growth performance and lowered blood cholesterol levels

(Ullah et al., 2023). Ultimately, the inclusion of soy lecithins in non-ruminant diets will largely be driven by price with the benefits in poultry outweighing the benefits in swine diets.

Spent bleaching clay and deodorizer distillate

Spent bleaching clay is the residual product of acid activated earths used to remove oxidation products, remaining soaps, and residual pigments. The quantity of earths or clays produced during bleaching will change depending on oil quality (Gerde et al., 2020). Additionally, spent bleaching earths are difficult to handle and are extremely flammable (Erickson, 1995c). Although steps are taken to minimize oil loss, some residual oil remains in the used earths. Some oilseed processors will add spent clays to soybean meal as a flow agent up to the allowable inclusion of 0.5% (Smallwood, 2015; NOPA, 2021). Furthermore, processors can slurry the spent bleaching earths with crude oil and add it back to the extractor to maximize the recovery of oil (Dijkstra, 2020). These options are limited to oil refineries that are located next to a soybean crushing plant. If the earths are not added back to the soybean meal, oil refineries must find another method of disposal. Although there are technologies that allow for regeneration of spent bleaching earths or development of lick blocks for cattle, these are still relatively uncommon (Smallwood, 2015; Dijkstra, 2020).

Smith (1984) observed that bleaching clays from canola processing could be fed to swine at up to 10% of the diet for 17 days without affecting growth performance or feed intake. Another study fed up to 7.5% spent bleaching clay from canola refining to broilers without negative effects on health or growth and observed a positive effect on pellet hardness (Blair et al., 1986). Although past literature does not report any negative effects of spent bleaching clays in swine or poultry diets, this research has been limited with most studies conducted more than 30 years ago. A review by Dijkstra (2020) summarized the best methods for handling spent

bleaching earths. In this review, it was mentioned that adding bleaching earths back to soybean meal or disposal in a landfill are the most common methods. Adding bleaching earths back to soybean meal is not advised as it will increase the ash content of the soybean meal, consequently affecting its quality. However, for integrated soybean crush facilities and oil refineries this is still the most economical option. Dijkstra (2020) advocates for the use of spent bleaching earths as a poultry feed ingredient when refineries are not adjacent to crush facilities. Further research is needed on how to best capture the energy value from this by-product for use in non-ruminant diets.

Deodorization is the final step in oil refining and removes volatile compounds using a distillate system to provide oil with a bland flavor, odor, and color. This process typically removes between 95 to 98% of tocophoerols and sterols in the final oil (Woerfel, 1995a). The by-product, deodorizer distillate, is valuable as it contains 4% tocopherol and 6% sterols (Gerde et al., 2020). Tocopherols are used as antioxidants and sterols are used to manufacture pharmaceuticals (Woerfel, 1995a). Due to their demand in human health and nutrition, these by-products are more valuable in those industries and are less likely to be added to the soybean meal to be used as animal feed.

CONCLUSION

Multiple by-products are produced during soybean processing and oil refining which must find a market or proper disposal method. These products can include weeds and foreign material, soybean hulls, gums, soapstocks, lecithin, spent bleaching clay, and deodorizer distillates. Limited information exists throughout the soybean processing industry on the composition and quantities produced of each by-product. While previous research has helped establish feeding values for some of these by-products, more up-to-date information is needed.

Both gums and soapstocks have shown a potential to act as an energy source in livestock diets, but there is limited information on the variation and quantities of gums and soapstocks produced and how they affect both poultry and swine performance. Therefore, these two soybean processing by-products have been identified as having the most potential value and need for additional research.

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Figure 4.1. Soybean processing by-products production throughout the soybean crushing and oil refining process.

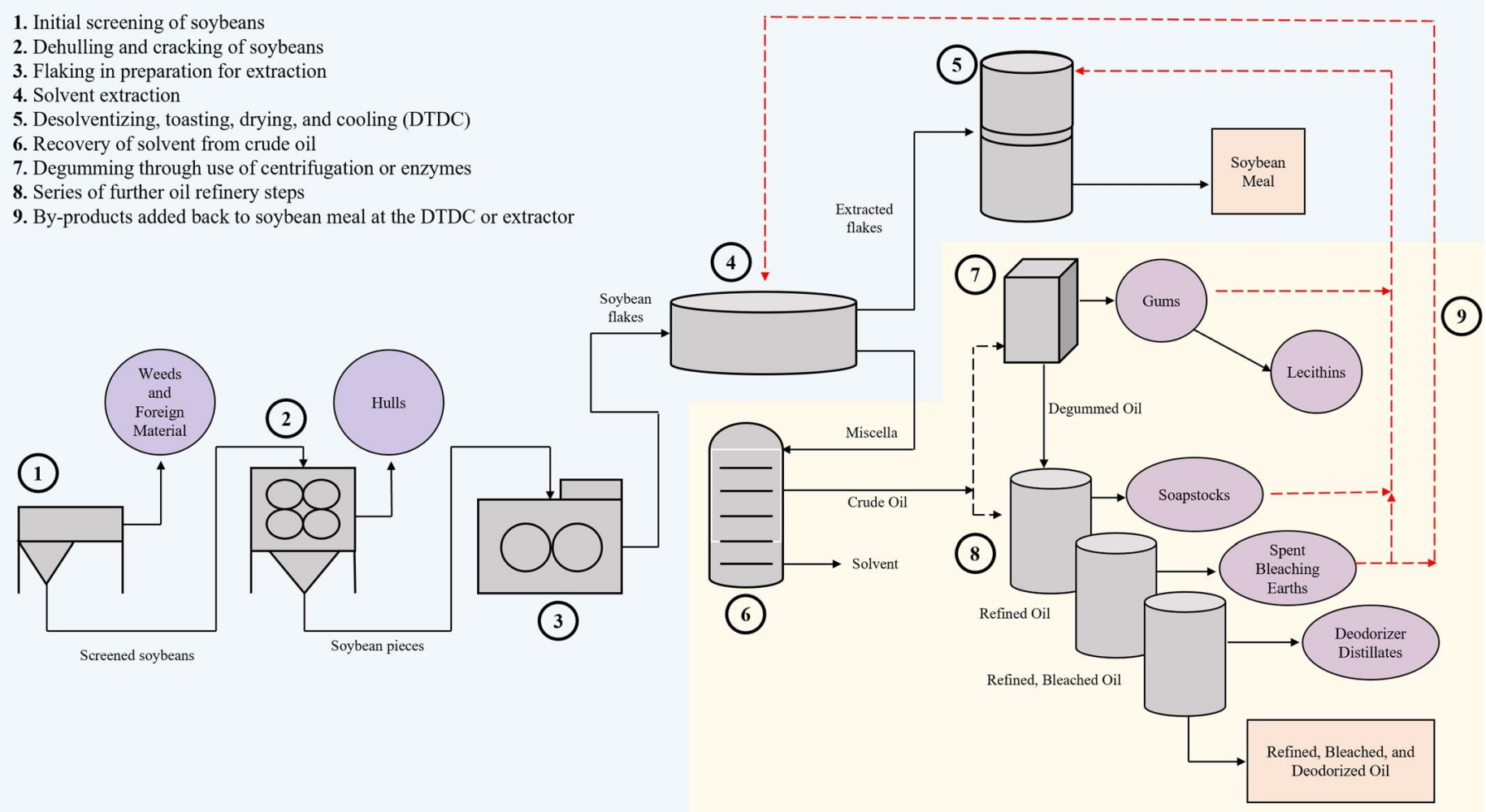

Table 4.1. Summary of soybean processing by-products and their use in monogastric diets.

Soybean processing by-product	Added back to soybean meal	Estimated amount added back to soybean meal	Common uses	Potential implementation in monogastric diets
Weeds and foreign material	No	---	Added to soybean hulls or disposed in landfill	None
Soybean hulls	No	---	Ruminant feedstuffs, wastewater treatment, and industrial applications	Dietary fiber source
Soybean gums	Yes	0 to 2%	Lecithin production or added back to soybean meal	Through soybean meal or as an energy source
Soybean soapstocks	Yes	0 to 2%	Acidulated for animal feed applications or added back to soybean meal	Through soybean meal, as an energy source, or as a pigment for poultry
Lecithins	No	---	Food applications, livestock feed, and pharmaceuticals	As an antioxidant or emulsification additive
Spent bleaching clays	Yes	Up to 0.50%	Added back to soybean meal as a flow agent, poultry feed ingredient, or disposal in landfill	Through soybean meal or poultry feed additive
Deodorizer distillates	No	---	Antioxidant or pharmaceutical applications	None (high demand in other markets)

Chapter 5 - An industry survey of the composition and variability of soybean gums and soapstocks across US soybean processing plants

ABSTRACT

Depending on the soybean processing plant, gums and soapstocks may be added back to soybean meal during soybean processing. Despite the potential effects on soybean meal quality, there is limited information on the composition and variation in soybean by-products and the resulting soybean meal if by-products are added back during processing. A total of 36 soybean by-product samples from 14 plants across 8 different companies were used in an industry survey evaluating the composition and variation of soybean gums and soapstocks across the US. All soybean processing plants within the study produced at least one of the two by-products: soybean gums or soybean soapstocks. Soybean by-product and soybean meal samples were collected at two different timepoints: May to July 2023 and October to November 2023. The individual plants surveyed constitute approximately 30% of total US soybean meal production, with the 8 participating companies representing 80% of the total US soybean meal production. By-products were analyzed for lipid quality criteria including moisture, fat by acid hydrolysis, fatty acid analysis, and oxidation markers. Furthermore, soybean meal samples were submitted for analysis of proximate composition, neutral detergent fiber, Ca, P, and trypsin inhibitor activity. Soybean gums had a greater ($P \leq 0.05$) percentage of acid hydrolyzed fat and p-Anisidine value compared to soybean soapstocks. Soybean soapstocks tended to have a greater ($P = 0.085$) percentage of moisture and volatile matter as well as an increased ($P = 0.052$) concentration of insoluble impurities compared with soybean gums. Most notably, there was considerable variation in the composition of by-product samples between processing plants indicating differences in processing procedures or incoming soybean quality. Soybean meal containing added soybean by-

products had 61% greater ($P < 0.05$) ether extract than soybean meal samples not containing added soybean by-products on a dry matter basis, but there was no difference ($P < 0.10$) in crude protein. Furthermore, trypsin inhibitor activity varied considerably between plants with values ranging from 1.45 to 9.26 TIU/mg of seed powder, regardless of by-product inclusion. These results provide information on the composition and variation in soybean by-products across various processing plants; however, further information is still needed to evaluate their subsequent effects on livestock diets.

INTRODUCTION

Soybean meal is an important, high quality protein source frequently used in livestock diets. In 2021, approximately 34 million metric tons of soybean meal were used in animal feeds, with 78% in poultry and swine diets (United Soybean Board, 2021). Therefore, understanding soybean meal composition, quality, and nutritive value is a crucial area of focus in monogastric nutrition. Despite the focus on soybean meal research, there has been limited attention on the composition and nutritional value of soybean processing by-products such as soybean gums and soapstocks. Although by-products of soybean oil refining, soybean gums and soapstocks have the potential to affect soybean meal quality. Soybean processors must find a market or method of utilizing each by-product produced throughout the process and one of the easiest and most affordable methods of managing soybean gums and soapstocks is by adding them back to soybean meal during processing (Gerde et al., 2020). This is most common when an oil refinery is adjacent to a soybean crushing facility (Figure 5.1).

Soybean gums are produced through the degumming step of oil refining in an effort to remove phosphatides from the oil, while soybean soapstocks are produced during caustic refining which removes any remaining phosphatides and neutralizes free fatty acids (Erickson,

1995a,b). If added back to soybean meal during processing, these by-products will be transferred to the desolventizing, toasting, drying, and cooling step of production to be dried along with soybean meal. These by-products have been suggested to contain approximately 35% crude oil (Erickson, 1995a,b). Therefore, adding these by-products back to soybean meal may have an effect on its quality.

The challenge with understanding how gums and soapstocks will affect soybean meal quality is that there is currently a lack of understanding on the composition of the soybean by-products themselves. Multiple factors can impact the composition of soybean by-products including initial soybean quality, degumming procedures, caustic refining procedures, age of machinery, and facility management (Erickson, 1995a,b; O'Brien, 2008). These factors likely lead to the composition of by-products varying across soybean processing plants. Therefore, the objective of this study was to investigate the composition and variability of soybean gums and soapstocks across US soybean processing plants and its impacts on subsequent soybean meal quality through an industry survey.

MATERIALS AND METHODS

A total of 36 soybean by-product samples from 14 soybean processing plants across 8 different companies were used in the industry survey. The individual plants surveyed constitute approximately 30% of total US soybean meal production, while the 8 participating companies represent 80% of the total US soybean meal production. All soybean processing plants within the study produced at least one of the two by-products: soybean gums or soybean soapstocks. Soybean by-product and soybean meal samples were collected at two different timepoints within the survey: May to July 2023 and October to November 2023. A total of 36 by-products samples were collected throughout the survey. By-product samples included 12 soybean gum and 6

soybean soapstock samples at each timepoint. Furthermore, a total of 26 soybean meal samples were collected with 7 soybean meal samples containing added by-products and 6 samples not containing added by-products at both sample collections. It is important to note that 2 plants within the survey only have samples represented at 1 timepoint as samples were not received by the deadlines set for this study.

Plants received six 500 mL bottles (three bottles for each by-product; Nalgene lab quality amber HDPE bottles, Thermo Scientific, Waltham, MA) and two 207 mL sampling bags (Whirl-Pak, Nasco, Ft. Atkinson, WI). Upon receipt of the sampling packages, an employee from each soybean plant collected the by-product samples applicable to their production system (soybean gums and/or soapstocks). Simultaneously, a soybean meal sample was collected from each plant. Information regarding the date of collection, employee name and contact, and if soybean processing by-products had been added back to soybean meal during the time of collection were recorded. Once collected, samples were immediately shipped overnight to Kansas State University to be prepared for analysis. All samples were stored at 4°C until analysis was performed.

Chemical analysis

Samples of soybean gums and soapstocks were submitted for lipid quality analysis (Table 5.1). Each by-product sample was homogenized at the laboratory prior to being analyzed. At both timepoints, the following criteria were measured in duplicate: moisture and volatile matter by hot plate (Method Ca 2b-38; AOCS, 2017 and Method Ba 2a-38; AOCS, 2022), insoluble impurities (Method Ca 3a-46; AOCS, 2021), unsaponifiable matter (Method Ca 6a-40; AOCS, 2017), P (Methods 984.27, 927.02, 985.01, and 965.17; AOAC, 1996), p-Anisidine value (Method Cd 18-90; AOCS, 2017), fat by acid hydrolysis (Method 954.02; AOAC, 1977), fatty

acid profile (Methods Ce 2-66 and Ce 1b-89; AOCS, 2017), and free fatty acids (Method 940.28; AOAC, 2012 and Method Ca 5a-40; AOCS, 2017).

Soybean meal samples were ground and analyzed in duplicate for Ca and P (Method 985.01 A, B, and D; Method 942.05; AOAC, 2006; Table 5.2). Soybean meal samples were also analyzed in duplicate for proximate composition including dry matter (DM; Method 930.15; AOAC, 1999), crude protein (Method 990.03; AOAC, 2002), ether extract (Method 2003.05; AOAC, 2006), crude fiber (Method Ba 6a-05; AOCS, 2017), and ash (Method 942.05; AOAC, 2005) as well as neutral detergent fiber (NDF) using Ankom technology (Method 2001.11; AOAC, 2005). A sample of soybean meal was submitted for analysis of trypsin inhibitor activity in triplicate utilizing procedures outlined by Kim and Krishnan (2023).

Statistical analysis

Data were analyzed using the lmer function from the lme4 package in R (version 2023.12.0 (2023-12-17), R Foundation for Statistical Computing, Vienna, Austria). For by-product analysis, soybean by-product type (soybean gums or soybean soapstocks) served as the fixed effect. For soybean meal analysis, soybean meal by-product inclusion (no by-products added, or by-products added) served as the fixed effect. Sample was included as a random effect for all criteria besides trypsin inhibitor activity to account for duplicate analysis. All results were considered significant with $P \leq 0.05$ and marginally significant with $P \leq 0.10$. Descriptive statistics were included to show the simple means of each analytical criteria within by-product or soybean meal type and timepoint of sample collection. Additionally, the range was included which represented the minimum and maximum values for each analytical criteria within the soybean by-product or soybean meal type and timepoint of sample collection. The range was

based on an individual analysis and does not represent the values of a sample analyzed in duplicate.

RESULTS

For the soybean by-product analysis (as-is), soybean gums had a greater ($P \leq 0.05$) percentage of acid hydrolyzed fat and p-Anisidine value compared to soybean soapstocks (Table 5.3). However, soybean soapstocks tended to have a greater ($P = 0.085$) percentage of moisture and volatile matter as well as an increased ($P = 0.052$) concentration of insoluble impurities compared to soybean gums. As a percentage of the extracted fat, soybean soapstocks tended to have increased ($P = 0.085$) concentrations of the fatty acid C18:1 which resulted in a tendency for increased ($P = 0.085$) total monounsaturated fatty acids (MUFA) compared to soybean gums. Inversely, as a percentage of the extracted fat, soybean gums tended to have increased ($P = 0.056$) concentrations of the fatty acid C18:2 which translated into a tendency for increased ($P = 0.082$) total polyunsaturated fatty acids (PUFA) compared to soapstocks.

For the soybean meal analysis, soybean meal containing added soybean by-products had a 61% increase ($P < 0.05$) in ether extract compared to soybean meal samples not containing added soybean by-products on a DM basis (Table 5.4). There was no evidence of differences ($P > 0.10$) in any other analytical criteria due to by-product inclusion.

For acid hydrolyzed fat (as-is; Figure 5.2) and moisture and volatile matter (as-is; Figure 5.3) content between processing plants, there was considerable variation regardless of soybean by-product type. Similarly, when examining the effect of soybean processing plant on soybean meal composition, there was considerable variation in ether extract (as-is); however, a large portion of the variation was driven by soybean by-product inclusion (Figure 5.4). Trypsin

inhibitor activity also varied considerably between plants with values ranging from 1.45 to 9.26 TIU/mg of seed powder (as-is; Figure 5.5).

DISCUSSION

Soybeans were first introduced to US agriculture in the 1770s, but soybean meal did not gain attention as a livestock feedstuff until the late 1930s (Ruiz et al., 2020). Since then, soybean meal has become an affordable, high-quality source of protein in monogastric diets. Despite the extensive research amassed on soybean meal quality, there has been limited focus on soybean processing by-products. These soybean processing by-products can include weeds and foreign material, soybean hulls, gums, soapstocks, spent bleaching clays, and deodorizer distillates. Although by-products of soybean processing and oil refining, soybean by-products have the potential to impact soybean meal quality because soybean by-products, such as soybean gums and soybean soapstocks, may be added back to soybean meal during processing ultimately influencing the final product (Gerde et al., 2020).

The addition of soybean processing by-products back to soybean meal is not novel with reports dating back to 1956 documenting this process (Brewster and Mitchell, 1956). For modern soybean meal production, soybean gums and soapstocks are the by-products most frequently added back to soybean meal during processing. Soybean gums are produced through the degumming step of oil refining to remove phosphatides from the oil (Erickson, 1995a), while soybean soapstocks are produced during caustic refining which removes any remaining phosphatides and neutralizes free fatty acids (Erickson, 1995b). When added back to soybean meal, these by-products will be transferred from the soybean oil refinery into the desolventizing, toasting, drying, and cooling step of production. This will allow the by-products to be dried along with the soybean meal and be fully incorporated into the final product.

Past literature has attempted to better understand the composition and utilization of gums and soapstocks. In The Practical Handbook of Soy Oil Processing and Utilization, Erickson (1995a) characterized the general composition of gums as 35% soybean oil, 17% phytogycolipids, 16% phosphatidyl choline, 14% phosphatidyl ethanolamine, 10% phosphatidyl inositol, and 7% carbohydrates. In a second chapter in the same book, Erickson (1995b) characterized soapstocks as 20 to 30% oil, 65 to 70% fatty acids, and 5% sterols and oxidized components. However, soybean meal production has continued to become more efficient and has adopted new technologies over the past 30 years; therefore, there is a need for updated information. Studies have assessed the potential value of utilizing soybean gums and soapstocks as feed ingredients in livestock diets (Bruce et al., 2006; Gaiotto et al., 2000; Rojas-Cano et al., 2014; Borsatti et al., 2018). These studies often did not characterize the composition of the by-products they were using, creating challenges when comparing the literature. In addition to needing a better understanding of the general composition, there is limited information on the amount of variation associated with these products. It is well documented that aspects including initial soybean quality, plant machinery, degumming processes, refining processes, and management can impact the quantities and composition of the by-products produced (Erickson, 1995a,b; Woerfel, 1995; O'Brien, 2008). Currently no studies quantify this variation.

In this industry survey, a total of 36 samples across 14 plants were collected. When categorized, 5 plants produced both soybean gums and soapstocks, 6 plants produced only soybean gums, and 2 plants produced only soybean soapstocks. This indicates that most oil refineries are choosing to utilize degumming, with only 2 plants not implementing degumming within their system at the time the sample collection occurred. Instead, approximately 36% of the plants sampled utilized both degumming and caustic refining, generating both soybean gums and

soapstocks. Interestingly, approximately 50% of the plants participating in the study utilized a degumming step likely in combination with physical refining, ultimately generating only gums. Unlike caustic refining, physical refining removes free fatty acids through steam injections and does not generate soapstocks (Gharby, 2022). Although not a comprehensive evaluation of all soybean processing plants across the US, this information gives current insight into soybean processing practices.

As expected, there were inherent differences in the composition of the soybean by-products. Most notably, soybean gums had 80% greater acid hydrolyzed fat content than soybean soapstocks on an as-is basis. This indicates that there may be more neutral oil loss during degumming than caustic refining. The quantity of neutral oil loss can largely be affected by the methods used during refining. For degumming, four method types may be used including water, acid, dry, or enzymatic degumming. Traditional methods of degumming, such as water degumming, typically have a higher oil loss compared to the lesser implemented, newer practice of degumming through enzymes (Dijkstra, 2010). Degumming is used to help prepare crude oil for either the caustic or physical refining step. If degumming is implemented, it removes phosphatides that negatively impact caustic refining, which allows the caustic refining step to be more efficient, resulting in lower oil loss (Woerfel, 1995).

In addition to the hydrolyzed fat content, the oil fatty acid content in the by-products tended to differ with soybean gums having greater concentrations of PUFA and soybean soapstocks having greater concentrations of MUFA. Although these differences are minimal, it may be driving the differences in the p-Anisidine value. Polyunsaturated fatty acids are more readily oxidized than MUFA leading to increased secondary oxidation products and subsequently an increased p-Anisidine value (Tao, 2015). Further investigation would be needed

to confirm that the p-Anisidine value is impacted by the fatty acid content and not related to aspects of the degumming process itself. Despite these differences, the fatty acid contents of both by-products closely reflect that of soybean oil (Dijkstra, 2016).

Besides differences in the fat measurements, soapstocks contained a greater percentage of insoluble impurities and tended to have increased moisture and volatile matter when compared to soybean gums. Insoluble impurities can include substances such as sand and debris, oxidized fatty acids, minerals, and alkali soaps (Eurolab, 2024). Inherently, caustic refining involves the removal of free fatty acids utilizing an alkali soap; therefore, it was expected that soapstocks would contain a greater percentage of insoluble impurities. Additionally, although there was a tendency for differences in moisture and volatile matter percentage between the two by-products, the interesting takeaway is the range in moisture content noted. Both soybean gums and soapstocks had an astounding amount of variation with ranges from 0.15 to 60.63% and 2.99 to 60.42%, respectively. This is important when considering the handling and transportation of the by-products as well as for potential implementation into animal diets.

In addition to by-products, a sample of soybean meal was collected from each plant. There was a total of 26 soybean meal samples collected across both timepoints. When categorized, there were 7 plants that reported adding soybean by-products back to the soybean meal and 6 plants that did not add by-products back during processing at each timepoint. Two plants changed their procedures between sampling timepoints and therefore are represented in both the no by-products added and the by-products added categories in Figures 5.4 and 5.5. Multiple factors can impact whether a plant will add soybean by-products back to soybean meal including if they have other marketing streams for the by-products or the initial incoming soybean quality.

For the soybean meal analysis, there was an increase in ether extract of soybean meal samples when soybean by-products were added back during processing. Woerfel (1995) suggested that the fat content of soybean meal increases by approximately 0.4 percentage units when soybean soapstocks are added back during processing. In alignment with previous literature, the current survey found a 0.7 percentage unit increase in fat content on a DM basis when soybean by-products, either gums or soapstocks, were added back. More specifically, soybean meal devoid of by-products had an ether extract range from 0.84 to 1.60% while soybean meal containing by-products had an ether extract range from 1.02 to 3.35% on a DM basis. Ultimately, if soybean meal is above approximately 1.60% (DM basis) ether extract, consumers can assume some level of soybean by-products have been added back during processing.

Besides the difference in ether extract, there were minimal differences in soybean meal composition. From a monogastric nutrition standpoint, the inclusion of soybean by-products may cause concerns of diluting the crude protein content of soybean meal. However, soybean processors only add by-products back at low levels and not at the detriment of meeting the crude protein specifications (NOPA, 2015). Furthermore, there may be some value to capture with soybean meal containing added by-products. With the current price of fat, soybean by-products may have the potential to serve as an affordable energy source. Therefore, additional research may be justified in evaluating the use of soybean meal containing added by-products on livestock diets.

An unintentional finding of this study was the level of trypsin inhibitor activity in the soybean meal samples. Trypsin inhibitor activity was evaluated on all soybean meal samples within this study as it serves as an important pillar of soybean meal quality. In the survey, the

average trypsin inhibitor activity was approximately 5.60 TIU/mg seed powder (as-is) with 73% of the soybean meal samples being above 4 TIU/mg of seed powder. High levels of trypsin inhibitor activity have been shown to negatively impact swine (Yen et al., 1974) and poultry (Erdaw and Beyene, 2018) performance. Therefore, the level of trypsin inhibitor activity in modern soybean meal production presents more of a concern to soybean meal quality than additions of soybean by-products during processing.

In summary, these data suggest soybean gums had a greater acid hydrolyzed fat content and a decreased moisture and volatile matter percentage than soybean soapstocks. Most notably, there was considerable variation in by-product composition between processing plants indicating differences in processing procedures or incoming soybean quality. When soybean by-products were added back to soybean meal, there was an increase in ether extract, but no effects on crude protein. Ultimately, soybean meal containing greater than approximately 1.6% ether extract on a DM basis likely contains soybean by-products. These results provide information on the current composition and variation of soybean by-products across various processing plants; however, further information is still needed to evaluate their subsequent impact on livestock diets.

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Figure 5.1. Soybean processing by-products production throughout the soybean crushing and oil refining process.

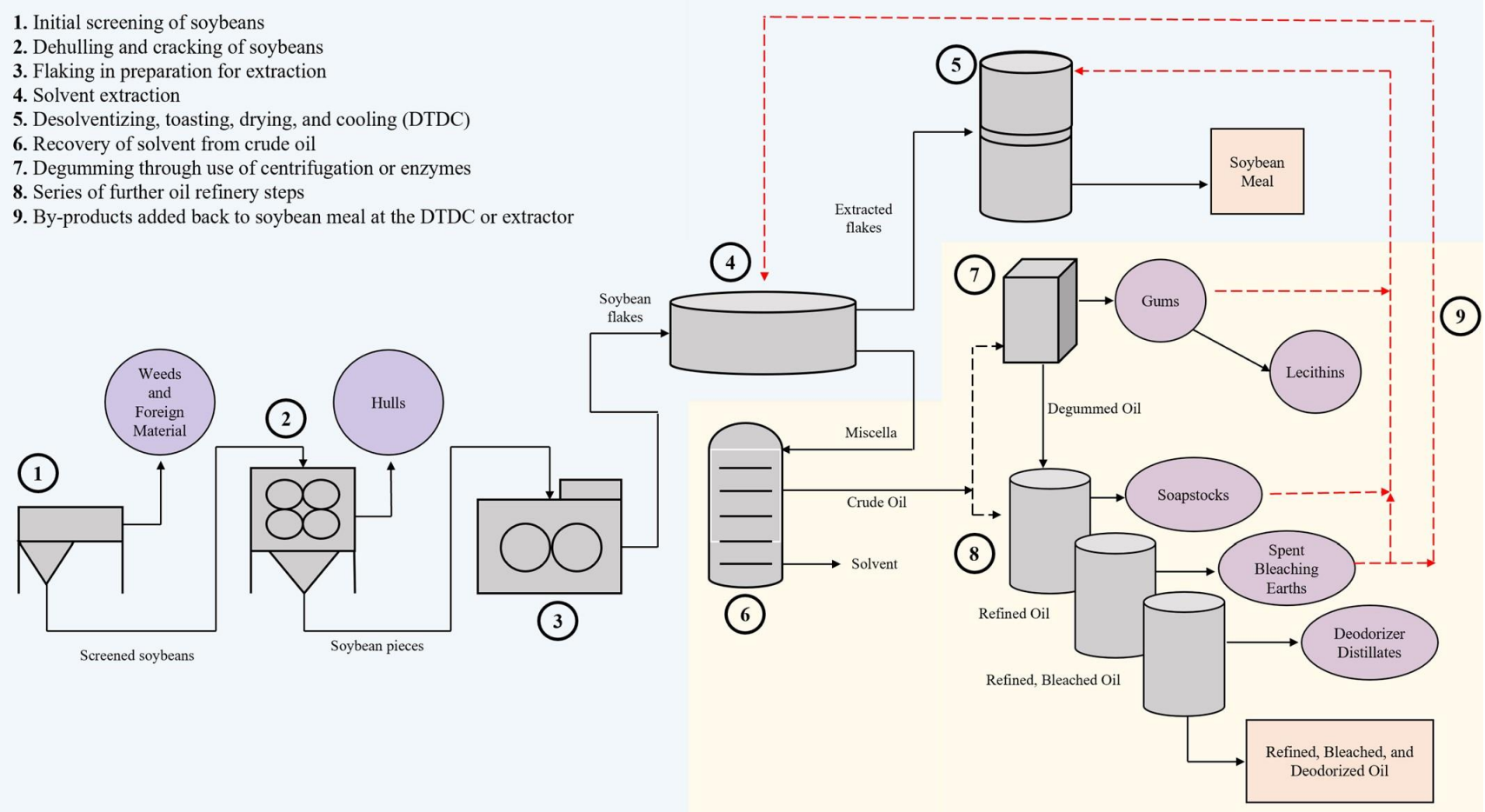

Figure 5.2. Fat by acid hydrolysis of soybean by-product samples by soybean processing plant and soybean by-product type (as-is)

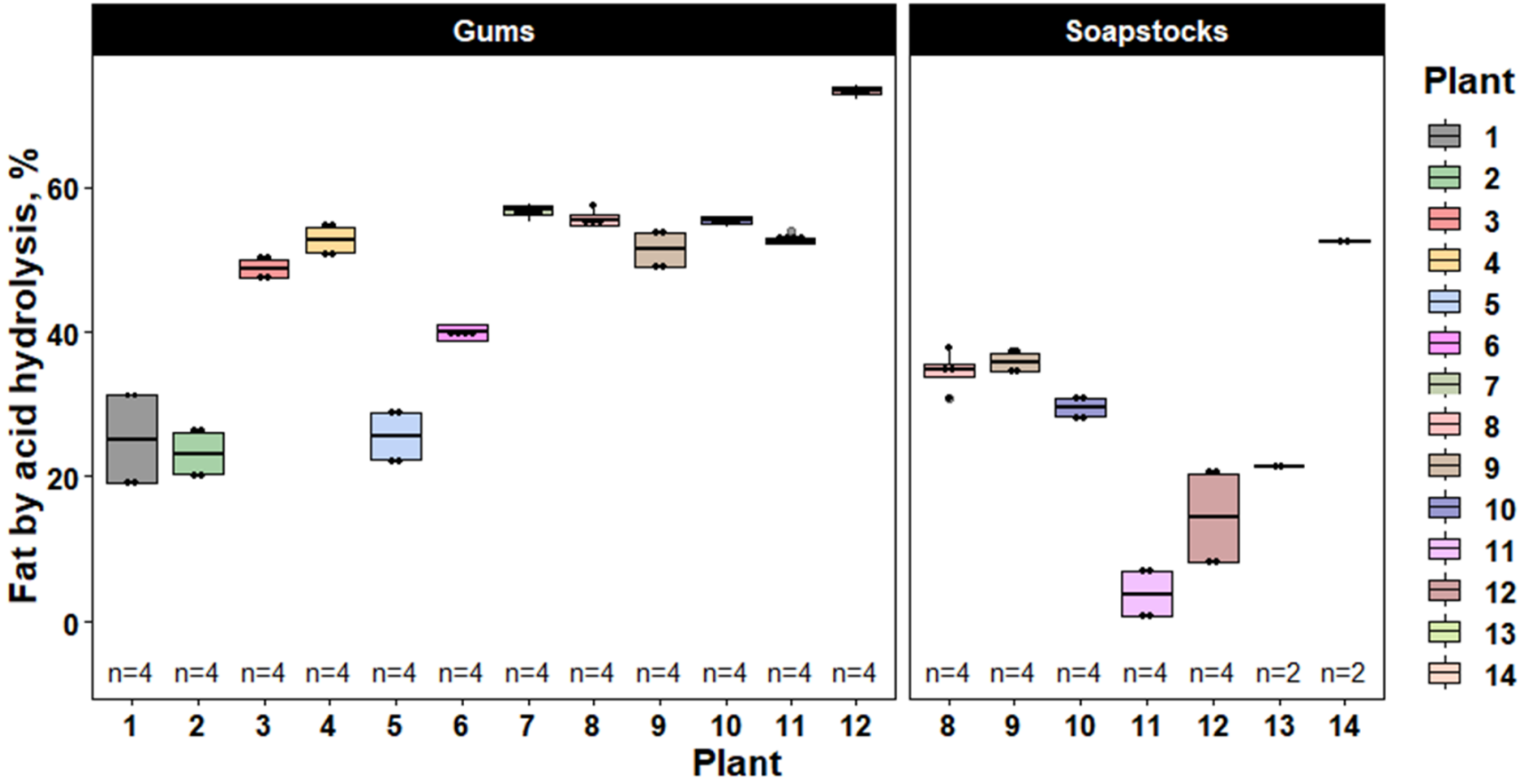

Figure 5.3. Moisture and volatile matter of soybean by-product samples by soybean processing plant and soybean by-product type (as-is)

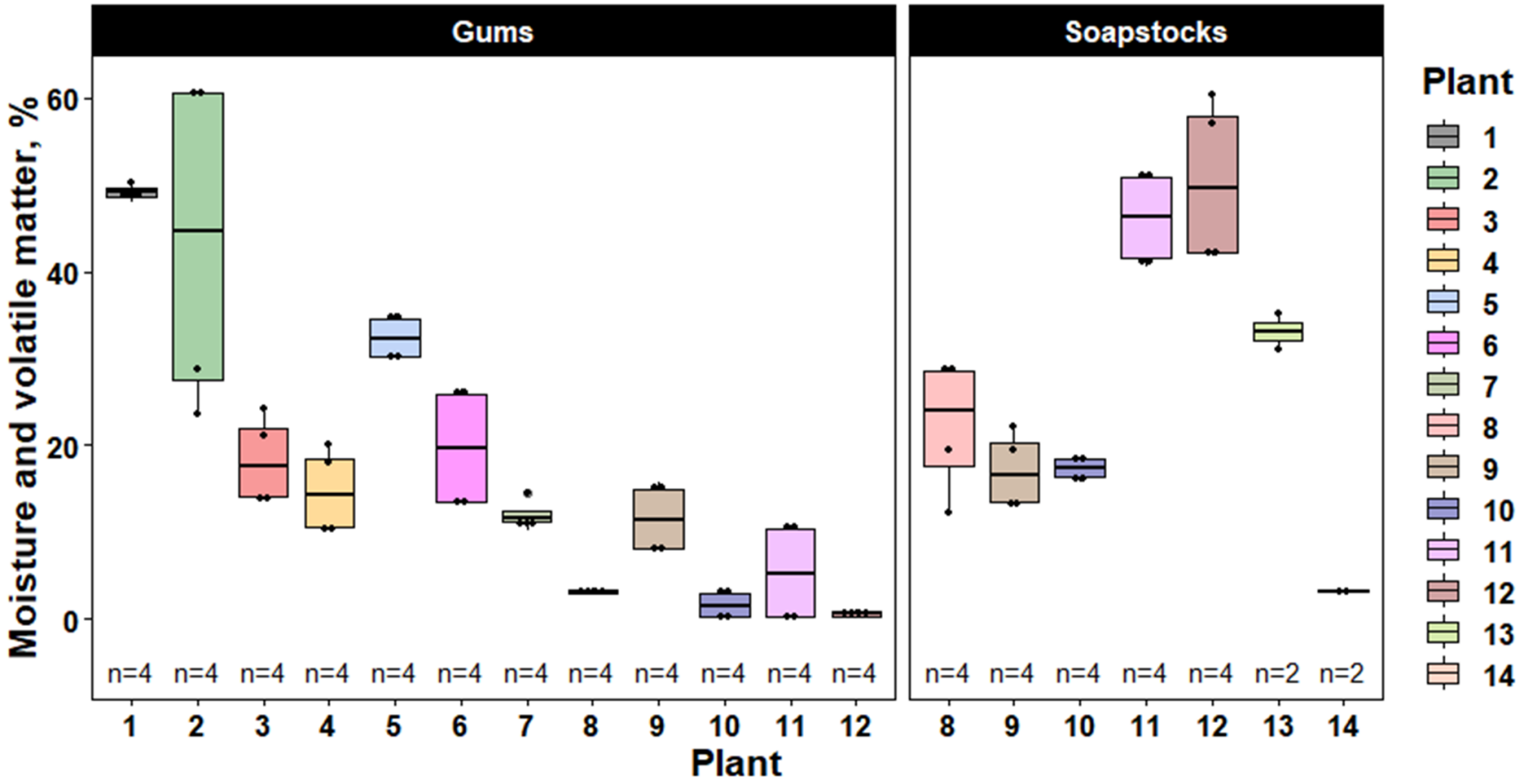

Figure 5.4. Ether extract of soybean meal samples by soybean processing plant and soybean by-product inclusion (as-is)

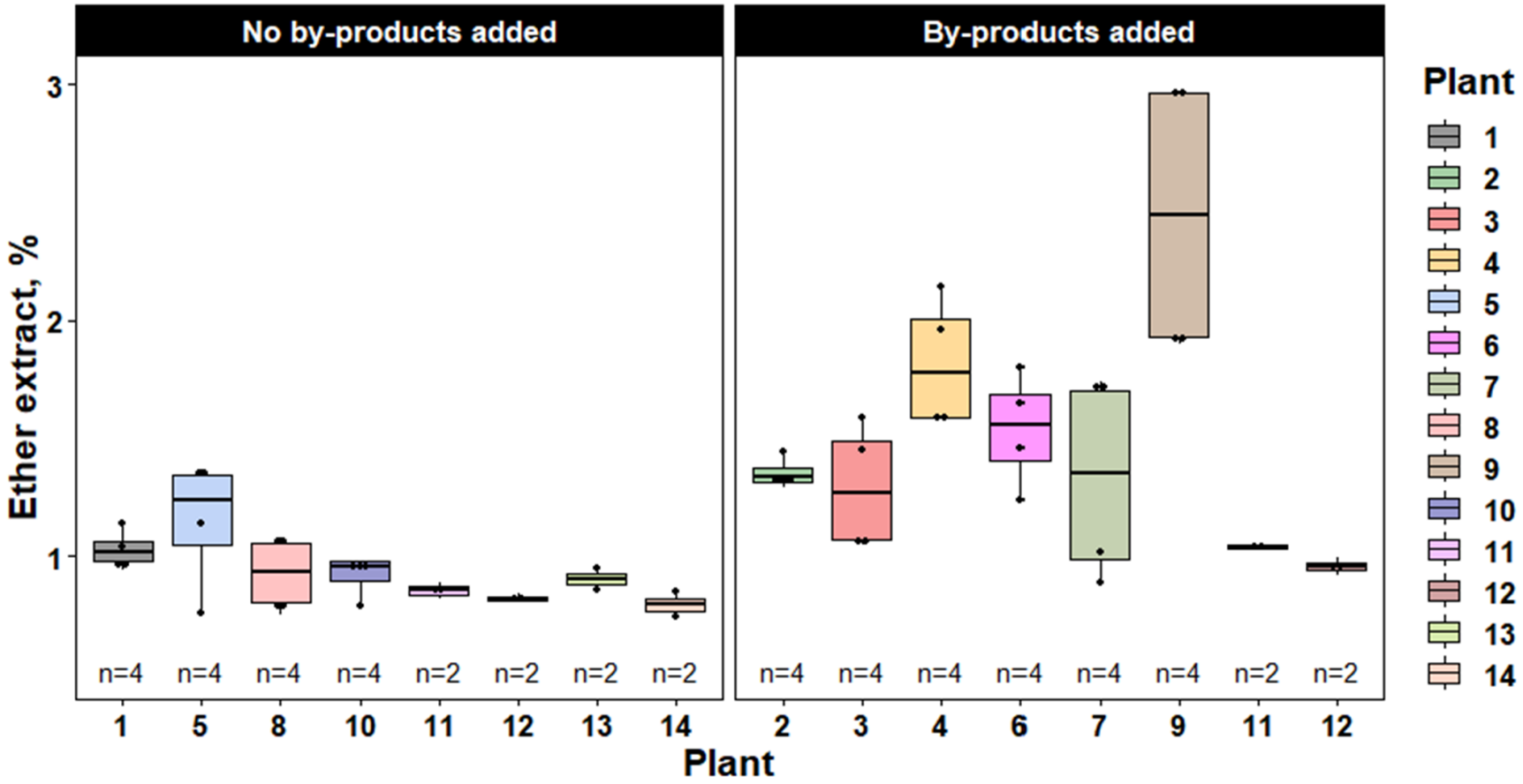

Figure 5.5. Trypsin inhibitor activity of soybean meal samples by soybean processing plant and soybean by-product inclusion (as-is)

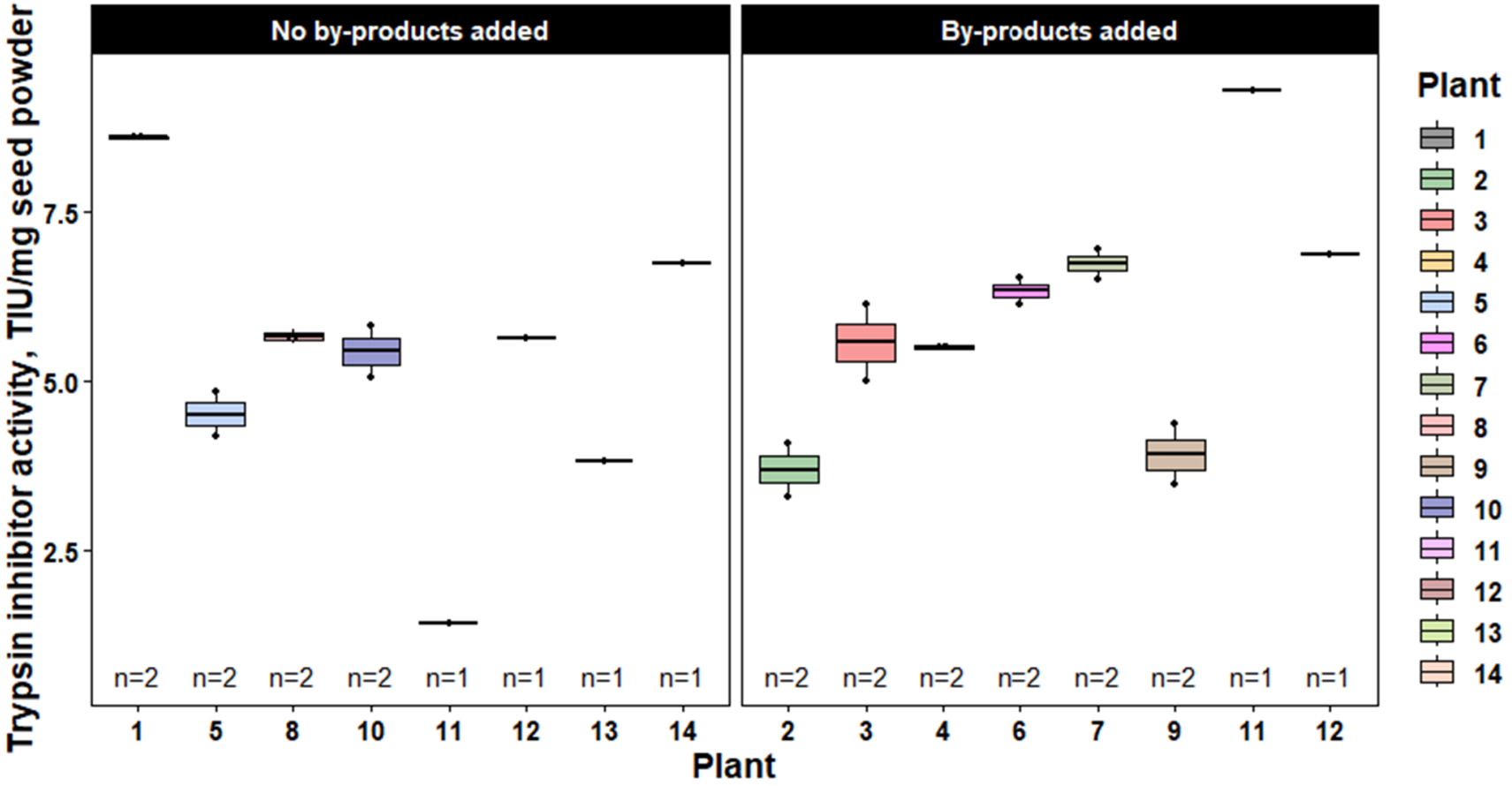

Table 5.1. Soybean by-product lipid quality analysis by by-product type and sampling timepoint (as-is)[1,2]

	Timepoint 1[3]				Timepoint 2			
	Gums		Soapstocks		Gums		Soapstocks	
Item	Mean	Range[4]	Mean	Range	Mean	Range	Mean	Range
Moisture and volatile matter, %	17.43	0.15-48.79	31.38	12.17-60.42	17.90	0.89-60.63	25.75	2.99-51.40
Insoluble impurities, %	0.26	0.05-0.86	2.85	0.14-6.82	1.60	0.09-6.84	14.42	0.00-46.78
Unsaponifiable matter, %	0.55	0.26-0.85	0.50	0.28-0.95	0.58	0.30-0.79	0.61	0.07-1.06
P, %	1.10	0.42-1.60	0.59	0.16-1.03	1.04	0.52-1.56	1.16	0.07-2.29
p-Anisidine value, %	22.9	1.3-58.9	2.1	0.0-4.1	3.3	0.0-19.4	2.4	0.0-4.6
Fat by acid hydrolysis, %	46.48	19.00-74.03	22.72	6.85-37.87	46.95	20.07-72.94	29.09	0.77-52.70
SFA, %[5]								
C16:0	14.76	10.53-16.27	14.22	11.97-16.25	14.51	11.31-15.79	13.92	9.52-17.23
C17:0	0.12	0.10-0.14	0.09	0.00-0.15	0.11	0.00-0.14	0.10	0.00-0.14
C18:0	4.26	3.81-4.84	4.40	3.35-5.16	4.22	3.55-5.17	4.15	3.57-4.82
C20:0	0.22	0.18-0.27	0.18	0.00-0.36	0.23	0.15-0.37	0.20	0.00-0.32
C22:0	0.41	0.37-0.52	0.48	0.37-0.74	0.45	0.35-0.92	0.37	0.00-0.50
C24:0	0.24	0.20-0.33	0.32	0.18-0.59	0.28	0.21-0.67	0.24	0.00-0.38
Total SFA	20.05	15.68-22.15	19.82	17.93-22.06	19.89	15.80-22.20	20.20	15.69-25.27
MUFA, %[5]								
C16:1	0.08	0.00-0.18	0.08	0.00-0.18	0.20	0.00-0.29	0.20	0.00-0.29
C18:1[6]	13.91	10.24-18.26	15.75	10.93-20.22	15.76	13.23-20.17	21.90	13.07-52.90
C20:1[6]	0.09	0.00-0.18	0.10	0.00-0.22	0.21	0.14-0.52	0.18	0.00-0.29
Total MUFA	14.11	10.34-18.49	15.93	10.93-20.48	16.22	13.69-20.66	22.31	13.61-52.90
PUFA, %[5]								
C18:2[6]	57.19	54.89-62.43	55.86	52.87-63.27	56.89	53.16-62.94	50.52	25.00-59.20
C18:3[6]	7.68	6.86-8.37	7.73	7.33-8.00	7.00	5.39-7.83	6.97	3.57-9.28
Total PUFA	64.87	61.75-69.94	63.58	60.74-71.14	63.90	60.11-69.29	57.50	28.57-67.22
Free Fatty acids, %[5]	9.69	3.14-15.91	7.10	0.16-21.40	10.14	6.20-33.00	7.58	2.40-20.80

[1]A total of 36 soybean by-product samples from 14 soybean processing plants across 8 different companies were used in an industry survey with 12 soybean gums and 6 soybean soapstocks submitted at each sampling timepoint.

[2]All samples were analyzed in duplicate.

[3]Samples were collected at two timepoints within the survey: May to July 2023 and October to November 2023.

[4]The range is the minimum and maximum value for each analytical criteria within soybean by-product type and timepoint of sample collection. Values represent an individual analysis and are not representative of the criteria in duplicate.

[5]Presented as a percentage of extracted lipid.

[6]Concentration includes isomers.

MUFA = monounsaturated fatty acids. PUFA = polyunsaturated fatty acids. SFA = saturated fatty acids.

Table 5.2. Soybean meal analysis by by-product inclusion and sampling timepoint (DM basis)[1,2]

	Timepoint 1[3]				Timepoint 2			
	No by-products added		By-products added		No by-products added		By-products added	
Item	Mean[4]	Range[5]	Mean	Range	Mean	Range	Mean	Range
DM, %	87.24	85.80-88.19	87.73	86.93-88.68	87.40	86.69-88.48	87.22	86.28-88.14
Crude protein, %	53.22 (46.43)	50.98-56.33	53.33 (46.79)	51.47-55.00	53.28 (46.57)	52.47-55.17	52.89 (46.14)	51.07-54.01
Ether extract, %	1.15 (1.00)	0.91-1.60	1.95 (1.72)	1.17-3.35	1.03 (0.90)	0.84-1.31	1.56 (1.36)	1.02-2.20
Crude fiber, %	5.99 (5.23)	4.01-7.39	5.89 (5.16)	4.41-8.35	4.58 (4.00)	3.53-5.26	4.87 (4.25)	3.95-6.15
Ash, %	6.62 (5.77)	6.05-7.98	6.65 (5.83)	6.02-8.13	6.63 (5.79)	6.23-7.09	6.68 (5.83)	6.24-7.27
Neutral detergent fiber, %	9.04 (7.88)	6.20-12.90	8.22 (7.21)	5.36-10.57	7.40 (6.47)	6.18-8.92	8.08 (7.04)	6.36-9.91
P, %	0.73 (0.64)	0.68-0.81	0.72 (0.63)	0.69-0.77	0.78 (0.68)	0.73-0.83	0.76 (0.67)	0.69-0.81
Ca, %	0.40 (0.35)	0.27-0.83	0.65 (0.57)	0.29-2.27	0.39 (0.34)	0.25-0.64	0.50 (0.44)	0.29-0.96
TIA, TIU/mg seed powder	6.31 (5.50)	4.33-9.81	6.52 (5.72)	3.79-10.57	6.30 (5.50)	1.67-9.85	6.49 (5.65)	3.94-7.98

[1]A total of 26 soybean meal samples were collected. At both timepoints, 7 soybean meal samples contained added by-products and 6 samples did not contain added by-products.

[2]All analyses besides trypsin inhibitor activity were run in duplicate. Trypsin inhibitor activity was analyzed in triplicate.

[3]Samples were collected at two timepoints within the survey: May to July 2023 and October to November 2023

[4]Analytical results are reported on a DM basis except for DM percentage. Values in parentheses represent the means on an as-is basis.

[5]The range is the minimum and maximum value for each analytical criteria within soybean meal by-product inclusion type and timepoint of sample collection. Values represent an individual analysis and is not representative of the criteria in duplicate.

DM = dry matter. TIA = trypsin inhibitor activity.

Table 5.3. Effects of soybean by-product type on lipid quality analysis (as-is)[1,2]

Item	Soybean by-product		SEM	*P* =
	Gums	Soapstocks		
Moisture and volatile matter, %	17.67	28.57	5.00	0.085
Insoluble impurities, %	0.93	8.63	2.975	0.052
Unsaponifiable matter, %	0.56	0.55	0.055	0.902
P, %	1.07	0.89	0.120	0.242
p-Anisidine value, %	13.09	2.23	3.692	0.021
Fat by acid hydrolysis, %	46.72	25.90	4.457	< 0.001
SFA, %[3]				
C16:0	14.64	14.07	0.525	0.386
C17:0	0.12	0.09	0.012	0.165
C18:0	4.24	4.28	0.125	0.810
C20:0	0.23	0.19	0.025	0.191
C22:0	0.43	0.43	0.037	0.973
C24:0	0.26	0.28	0.030	0.714
Total SFA	19.97	20.01	0.566	0.953
MUFA, %[3]				
C16:1	0.14	0.14	0.020	0.935
C18:1[4]	14.83	18.83	1.834	0.085
C20:1[4]	0.15	0.14	0.022	0.658
Total MUFA	15.16	19.12	1.817	0.085
PUFA, %[3]				
C18:2[4]	57.04	53.19	1.588	0.056
C18:3[4]	7.34	7.35	0.228	0.964
Total PUFA	64.39	60.54	1.750	0.082
Free Fatty acids, %[3]	9.91	7.34	1.886	0.267

[1]Samples were collected at two timepoints within the survey: May to July 2023 and October to November 2023. All samples were analyzed in duplicate.

[2]A total of 24 soybean gums and 12 soybean soapstocks were analyzed at two different timepoints from 14 soybean processing plants across 8 different companies.

[3]Presented as a percentage of extracted lipid.

[4]Concentration includes isomers.

MUFA = monounsaturated fatty acids. PUFA = polyunsaturated fatty acids. SFA = saturated fatty acids.

Table 5.4. Effects of soybean meal by-product inclusion on soybean meal composition, DM basis[1,2]

	Soybean meal by-product inclusion			
Item	No by-products added[3]	By-products added	SEM	*P* =
DM, %	100 (87.32)	100 (87.48)	0.158	0.472
Crude protein, %	53.25 (46.50)	53.11 (46.46)	0.331	0.764
Ether extract, %	1.09 (0.95)	1.76 (1.54)	0.132	0.001
Crude fiber, %	5.28 (4.61)	5.38 (4.71)	0.226	0.760
Ash, %	6.63 (5.79)	6.65 (5.82)	0.122	0.803
Neutral detergent fiber, %	8.22 (7.18)	8.15 (7.13)	0.268	0.846
P, %	0.76 (0.66)	0.74 (0.65)	0.009	0.198
Ca, %	0.40 (0.35)	0.58 (0.51)	0.108	0.232
TIA, TIU/mg seed powder	6.31 (5.51)	6.50 (5.69)	0.565	0.815

[1]Samples were collected at two timepoints within the survey: May to July 2023 and October to November 2023. All analyses besides trypsin inhibitor activity were run in duplicate. Trypsin inhibitor activity was analyzed in triplicate.

[2]A total of 26 soybean meal samples were collected. At both timepoints, 7 soybean meal samples contained added by-products and 6 samples did not contain added by-products.

[3]Analytical results and statistics are reported on a DM basis besides DM percentage which is on an as-is basis. Values in parentheses represent the model adjusted means on an as-is basis calculated by utilizing the treatment analyzed dry matter percentage.

DM = dry matter. TIA = trypsin inhibitor activity.

Chapter 6 - Effects of soybean gums and soybean soapstocks on weanling pig growth performance, fecal dry matter, and apparent total tract digestibility of dry matter

ABSTRACT

The soybean processing industry has not fully characterized the nutritional value of certain processing by-products and their effects on soybean meal (SBM) quality. Depending on the plant, gums and soapstocks may be added back to SBM. There is potential for these by-products to serve as an affordable energy source for swine due to their residual oil content. A total of 350 pigs (Line 241 × 600, DNA; initially 5.3 ± 0.02 kg) were weaned at approximately 19 d of age and used in a 42-d experiment. At weaning, pigs were randomly assigned to pens and allotted to 1 of 5 dietary treatments. There were 5 pigs per pen and 14 pens per treatment. Diets were fed in 3 phases: phase 1 from weaning to d 11, phase 2 from d 11 to 23, and phase 3 from d 23 to 42. Treatments included a control diet containing SBM with no added soybean by-products. Two additional diets contained gums or soapstocks at 4% of the SBM level in the diet. Another treatment diet contained SBM with 2% added soybean gums and 2% added soybean soapstocks. Lastly, a negative control contained 4% less SBM with no added by-products to have equal protein from soybean meal as the diets with added gums or soapstocks. Feces were collected on d 11 and 23 from 3 pigs per pen to determine fecal dry matter (DM). Fecal samples on d 23 were used to determine apparent total tract digestibility (ATTD) of DM. From d 0 to 11 (phase 1) and d 11 to 23 (phase 2), there was no evidence of differences ($P > 0.10$) for any growth response criteria. From d 23 to 42 (phase 3), pigs fed SBM with added gums had increased ($P = 0.05$) average daily gain (ADG). However, there was no evidence for differences

($P > 0.10$) in average daily feed intake (ADFI) or gain-to-feed ratio (G:F). For the overall experimental period (d 0 to 42), there was no evidence of differences ($P > 0.10$) due to dietary treatment for ADG, ADFI, or G:F. Fecal DM was approximately 19% on both d 11 and 23 and was not affected ($P > 0.10$) by treatment. There was an interaction ($P = 0.019$) between soybean gum and soapstocks for the ATTD of DM. When adding 4% gums to the diet, there was an improvement in the ATTD of DM; however, there was no evidence of differences when adding 4% soapstocks or 2% gums and 2% soapstocks to SBM. These data suggest that adding soybean processing by-products had minimal effects on nursery pig growth performance. However, there is a potential for improved ADG in the late nursery period when SBM containing added gums is included in the diet.

INTRODUCTION

Soybean meal is a fundamental amino acid source in swine diets, and as a result, soybean meal processing and its effects on pig growth performance has been extensively outlined in past literature. Depending on the processing plant, processing by-products such as soybean gums and soapstocks may be added back to soybean meal during processing (Gerde et al., 2020). This is most common when an oil refinery is located next to a soybean crushing plant. Soybean gums are produced through the degumming step of oil refining in order to remove phosphatides from crude soybean oil (Erickson, 1995a,b) while soybean soapstocks are produced during the caustic refining step utilizing sodium hydroxide in an effort to neutralize free fatty acids (Erickson, 1995a,c). There is potential for these by-products to serve as an affordable energy source for swine due to their residual oil content. However, they may also contain impurities removed from crude oil during refining.

The Practical Handbook of Soybean Processing and Utilization (1995) reported the general composition of soybean gums as 35% soybean oil, 17% phytogycolipids, 16% phosphatidyl choline, 14% phosphatidyl ethanolamine, 10% phosphatidyl inositol, and 7% carbohydrates (Erickson, 1995b) and soybean soapstocks as 20 to 30% oil, 65 to 70% fatty acids, and 5% sterols and oxidized components (Erickson, 1995c). However, the quality of incoming soybeans, machinery, manufacturing processes, and management can vary greatly between soybean processing facilities leading to substantial variation in the composition and quantities of by-products produced. Therefore, it is currently difficult to define how these by-products will impact soybean meal quality when added back during processing.

Although the literature on the effects of soybean by-products in swine diets is limited, a few studies have investigated the utilization of gums and soapstocks. Studies utilizing soapstocks in finishing pig diets found a potential for improved average daily gain (ADG) and feed-to-gain ratio (G:F; Lazor and Biocanin, 1970; Rojas-Cano et al., 2014) largely driven by the increase in energy density of the diets. However, these studies have limitations including being derived from olive oil (Rojas-Cano et al., 2014) and sunflower milling (Lazor and Biocanin, 1970), not soybean processing. Additionally, these studies evaluated soapstocks as a feed ingredient added to a basal diet, instead of evaluating the effects when added back to meal directly. One previous study by Bruce et al. (2006) aimed to characterize the effects of soybean by-products on digestibility, specifically when added back to soybean meal. However, this study was not designed to evaluate growth performance and is not representative of the soybean meal (lower crude protein and higher ether extract) currently produced throughout the industry. There is currently limited literature evaluating the use of these by-products during the nursery period, the time when a pig is most susceptible to changes in soybean meal quality. Therefore, the objective

of this study was to investigate the effects of utilizing soybean meal containing added soybean gums and soybean soapstocks on the growth performance, fecal dry matter (DM), and apparent total tract digestibility (ATTD) of DM for nursery pigs.

MATERIALS AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Each pen contained a 4-hole, dry self-feeder and nipple water. Pigs were provided *ab libitum* access to feed and water throughout the trial.

A total of 350 pigs (DNA 241 × 600, DNA; initially 5.3 ± 0.02 kg) were weaned at approximately 19 d of age and randomly assigned to pens then allotted to 1 of 5 dietary treatments. There were 5 pigs per pen (3 barrows and 2 gilts or 2 barrows and 3 gilts) and 14 pens per treatment. Treatments included a control diet containing soybean meal with no added soybean by-products. Two additional diets contained gums or soapstocks added at 4% of the soybean meal level in the diet. Another treatment diet contained soybean meal with 2% added soybean gums and 2% added soybean soapstocks. Lastly, a negative control contained 4% less soybean meal with no added by-products to have equal soybean protein as diets with added gums or soapstocks.

All soybean meal was sourced as a single batch for this trial and contained no added soybean by-products during manufacturing (Bunge North America, Emporia, KS). Similarly, a single batch of each by-product was sourced and utilized across all three dietary phases. Diets were manufactured at O.H. Kruse Feed Technology Innovation Center at Kansas State University, Manhattan, KS. Prior to mixing treatment diets, soybean meal was mixed with soybean by-products to provide four batches of soybean meal: soybean meal containing no by-

products, soybean meal containing 4% soybean gums, soybean meal containing 4% soybean soapstocks, and soybean meal containing 2% soybean gums and 2% soybean soapstocks. Treatment diets were then mixed utilizing the proper soybean meal sublots (Table 6.1). For diets containing soybean by-products and the diet containing 4% less soybean meal, increased quantities of feed grade amino acids were added to maintain similar dietary amino acid levels across all treatment diets. Nutrients for all treatment diets were formulated to meet or exceed the NRC (2012) requirement estimates for nursery pigs in each appropriate weight range. During bagging, complete diet samples were collected from every fourth bag, pooled, ground to reduce particle size, and stored at -20°C.

Pig weights and feed disappearance were measured on d 0, 11, 18, 23, 32, and 42 to determine ADG, average daily feed intake (ADFI), and G:F. All diets were fed in meal form in 3 phases: phase 1 from weaning to d 11, phase 2 from d 11 to 23, and phase 3 from d 23 to 42. Feces were collected on d 11 and d 23 from 3 pigs per pen to determine fecal dry matter. Additionally, titanium dioxide was included in phase 2 diets as an indigestible marker to determine apparent total tract digestibility (ATTD) of DM from fecal samples collected on d 23.

Chemical analysis

Soybean meal and complete diet samples were ground and analyzed for duplicate analysis of Ca and P (Method 985.01 A, B, and D; Method 942.05; AOAC, 2006; Table 6.2) at the K-State Research and Extension Soil Testing Laboratory in Manhattan, KS. Samples were also submitted to Midwest Laboratories Inc., Omaha, NE for duplicate analysis of proximate composition including dry matter (Method 930.15; AOAC, 1999), crude protein (Method 990.03; AOAC, 2002), ether extract (Method 2003.05; AOAC, 2006), crude fiber (Method Ba

6a-05; AOCS, 2017), and ash (Method 942.05; AOAC, 2005) as well as neutral detergent fiber using Ankom technology (Method 2001.11; AOAC, 2005).

Samples of soybean gums and soapstocks were submitted for lipid quality analysis at Eurofins Nutrition Analytical Center (Des Moines, IA; Table 6.3). For both by-product samples, the following criteria were measured in quadruplet: moisture and volatile matter by hot plate (Method Ca 2b-38; AOCS, 2017), insoluble impurities (Method Ca 3a-46; AOCS, 2021), unsaponifiable matter (Method Ca 6a-40; AOCS, 2017), P (Methods 984.27, 927.02, 985.01, and 965.17; AOAC, 1996), peroxide value (Method Cd 8-53; AOCS, 2003), p-Anisidine value (Method Cd 18-90; AOCS, 2017), fat by acid hydrolysis (Method 954.02; AOAC, Baur and Ensminger, 1977), fatty acid profile (Methods Ce 2-66 and Ce 1b-89; AOCS, 2017), and free fatty acids (Method 940.28; AOAC, 2012). Additionally, viscosity was analyzed in duplicate using the Brookfield procedure (Method Ja 10-87; AOCS, 2017).

Digestibility analysis

At the conclusion of the study, fecal samples were dried at 55°C for 48 h. The loss of weight was used to calculate fecal DM percent. Following fecal DM determination, both ground feed and fecal samples were dried in a 135°C forced air drying oven for 2 h to determine percentage DM of the samples used for titanium analysis (Method 985.01; AOAC, 2007). Titanium dioxide concentration in both dried feed and fecal samples were determined utilizing procedures outlined by Leone (1973). The ATTD of DM was determined using the index method described by Adeola (2001) using the following equation:

$$ATTD\ DM, \% = \left[1 - \left[\left(\frac{DM, \%_{Fecals}}{DM, \%_{Feed}}\right) \times \left(\frac{\mathrm{TiO_2}, \%_{Feed}}{\mathrm{TiO_2}, \%_{Fecals}}\right)\right]\right] \times 100$$

Statistical analysis

Data were analyzed as a completely randomized design for a one-way ANOVA using the GLIMMIX procedure of SAS v. 9.4 (SAS Institute, Inc., Cary, NC). Pen served as the experimental unit and treatment served as the fixed effect. In addition to the evaluation of treatment effect, the control treatment and treatments containing added gums and/or soapstocks were evaluated as a 2 × 2 factorial to investigate the interaction between added gums and soapstocks, as well as main effect of the addition of each by-product. Similarly, a contrast statement was used to compare pigs fed the diet containing soybean meal without soybean by-products and the diet containing 4% less soybean meal. Furthermore, contrasts were used to test for the main effects of treatment, day, and interaction between treatment and day for fecal DM. Results were considered significant with $P \leq 0.05$ and marginally significant with $P \leq 0.10$.

RESULTS

Lipid quality analysis of soybean gums and soapstocks sourced for this trial showed considerable differences in acid hydrolyzed fat, moisture and volatile matter, free fatty acids (FFA), P concentrations, and viscosity between the two by-products on an as-is basis (Table 6.3). Soybean gums had a greater percentage of acid hydrolyzed fat (52.3% vs 6.5%) and FFA (12.8% vs 0.5%) compared to soybean soapstocks. Inversely, soapstocks had a greater moisture and volatile matter content (44% vs 20%) which was then reflected in the viscosity of the by-products with soybean soapstocks having a lower viscosity than soybean gums. Finally, soybean gums contained approximately 0.95% P compared to soapstocks which contained only 0.16% P.

From d 0 to 11 (phase 1) and d 11 to 23 (phase 2), there was no evidence of differences ($P > 0.10$) for any growth response criteria (Table 6.4). From d 23 to 42 (phase 3), feeding soybean meal with added gums increased ($P = 0.053$) ADG. However, there was no evidence for

differences ($P > 0.10$) in ADFI or G:F. For the overall experimental period (d 0 to 42), there was no evidence of differences ($P > 0.10$) due to dietary treatment for ADG, ADFI, or G:F.

There was no evidence ($P = 0.775$) of an interaction between treatment and day for fecal DM. Furthermore, there was no evidence of a main effect of treatment ($P = 0.862$) or day ($P = 0.375$) with pigs exhibiting fecal DM of approximately 19% for both sampling timepoints.

There was an interaction ($P = 0.019$) between soybean gum and soapstocks for ATTD of DM. When adding 4% gums to the diet, there was an improvement in the ATTD of DM compared to the control diet; however, there was no evidence of differences when adding 4% soapstocks or 2% gums and 2% soapstocks to soybean meal compared to the control diet.

DISCUSSION

Soybean meal serves as an important source of high-quality protein in animal feeds, with approximately 78% of the total soybean meal produced going towards poultry and swine diets (United Soybean Board, 2021). Although a large portion of soybean meal is allocated to the livestock industry, soybean processing is driven by the oil processing industry with high demand in both human consumption and industrial applications (Shurtleff and Aoyagi, 2004). Depending on the desired product, crude soybean oil can go through multiple refining steps including degumming, neutralization, bleaching, and deodorization to yield a final, fully refined product (Erickson, 1995a). Throughout each step of the soybean crushing and oil refining process, by-products are produced in addition to soybean meal and soybean oil. These by-products can include weeds and trash, soybean hulls, gum, lecithins, soapstocks, spent bleaching clay, and deodorizer distillates. Once produced, by-products may be disposed of as waste, sold to an identified market to add value, or added back to soybean meal.

Both soybean gums and soapstocks are by-products specifically produced during the early stages of refining crude soybean oil. Soybean gums are produced through the degumming step of oil refining in order to remove phosphatides from crude soybean oil (Erickson, 1995a,b). Soybean soapstocks are produced during the caustic refining step utilizing sodium hydroxide in an effort to neutralize free fatty acids (Erickson, 1995a,c). Depending on the processing plant, soybean gums and soapstocks may be added back to soybean meal during processing (Gerde et al., 2020). This is most common when an oil refinery is located next to a soybean crushing plant. Decisions pertaining to by-product handling is heavily driven by economics as including by-products back to the soybean meal is almost always more economical than the cost of transportation or disposal. Soybean gums and soapstocks will often be added into the desolventizing, toasting, drying, and cooling (DTDC) step of soybean meal manufacturing. This allows the by-products to be dried down and fully incorporated. Currently, the quantities of by-products being added back to soybean meal is not clear; however, it has been estimated to range from 0 to 2% (Gaffield et al., 2024a).

When highlighting the lipid quality analysis for the soybean gums and soapstocks sourced for this trial, it is clear that there are inherent differences between the two by-products. One pronounced difference is the crude fat content. Based on procedures used to refine oil, it is expected that there would be some residual crude oil residing in the by-products. In this study, soybean gums were approximately 50% crude fat by acid hydrolysis while soapstocks contained minimal amounts with approximately 6% crude fat on an as-is basis. This stark difference in fat content was reflected when integrated back into the soybean meal. Soybean meal mixed with 4% gums had a greater ether extract compared to the control soybean meal containing no additional by-products (4% vs. 1%) on an as-fed basis. This increase in lipid content of soybean meal

containing added by-products was also observed by Gaffield et al. (2024b). In a survey of 14 soybean processing plants across the United States, soybean meal samples containing added soybean by-products had a greater ether extract percent than soybean meal samples devoid of soybean processing by-products (Gaffield et al., 2024b).

The increase in lipid content when utilizing gums may partially explain the improvement in ADG seen during the late nursery period. This improvement in gain coincides with when diets contained the greatest amount of soybean meal and therefore the greatest amount of gums. Previous studies evaluating the use of by-products including olive oil soapstocks at 7.5% of the diet (Rojas-Cano et al., 2014) and sunflower soapstocks at 2% of the diet (Lazor and Biocanin, 1970) observed improvements in ADG and feed efficiency in finishing pigs. In the present study, there was no reduction in feed intake and in return, no improvement in feed efficiency as commonly seen when including increased levels of fat or oil in the diet. However, the addition of these by-products in the present study are low and only comprised approximately 1% of the complete phase 3 diet. These levels were chosen in an effort to be representative of the amounts commonly added back to soybean meal and subsequently reflect the concentrations that would be formulated into a nursery diet. Therefore, the inclusion level was likely not great enough to influence feed efficiency unlike the previous studies.

In addition to the increase in crude fat adding energy to the diet, the improvement in ADG may also be affected by other factors. Pettigrew and Moser (1991) listed the two main objectives for including fat in nursery pig diets which were (1) improved gain and feed efficiency and (2) improved palatability. A study by Leibbrandt et al. (1975) observed that weanling pigs preferred diets containing animal or hydrolyzed fats compared to diets without added fat. Although there were no statistical differences in ADFI, the present study observed that

diets containing gums had a numerically greater ADFI throughout all three phases compared to diets containing soapstocks or diets without by-products. Therefore, palatability may be a potential driver of the improvement in ADG noted in this study during phase 3. However, research utilizing by-products in a preference study would be needed to validate the effects of soybean gums or soapstocks on the palatability of swine diets, specifically in the nursery period.

In addition to palatability, digestibility may be another factor affected by the inclusion of soybean by-products back to soybean meal. In the present study, adding 4% gums to the diet improved the ATTD of DM; however, there was no differences in digestibility when adding 4% soapstocks or 2% gums and 2% soapstocks to soybean meal. This contradicts the previous literature evaluating the digestibility of diets containing gums and soapstocks. There were no differences in ATTD of protein, fat, Ca, Mg, or P when feeding soybean soapstock up to 10% of the diet (Atteh and Leeson, 1985) or ATTD of DM, crude protein, ether extract, and gross energy when feeding olive oil soapstock at 7.5% of the basal diet (Rojas-Cano et al., 2014). However, these studies utilize by-products at a much greater inclusion level and in older pigs than the present study. Furthermore, it is challenging to characterize the differences in the composition of the actual by-products sourced as the composition changes greatly between plants and over time (Gaffield et al. 2024b).

Bruce et al. (2006) evaluated the effects of soybean by-products on the digestibility of soybean meal. In that study, they observed that pigs fed soybean meal with increasing concentrations of gums or soapstocks had a decrease in the apparent ileal digestibility (AID) of DM. This is inverse to the results observed in the present study where the ATTD of DM was improved when adding 4% gums but not when adding 4% soapstocks. This comparison is interesting as these two studies are the only trials evaluating soybean gums and soapstocks when

specifically added back to soybean meal. Additionally, the inclusion levels of by-products are similar, with Bruce et al. (2006) utilizing 1 to 3% gums and 0.5 to 1.5% soapstocks while the present study utilized 4% of soybean by-product or 2% of each by-product when combined. However, one distinct difference between the two studies is the composition of the initial soybean meal. The soybean meal sourced for the control diet in the present study had approximately 3% greater crude protein and 2.5% less ether extract on a DM basis than the soybean meal sourced for the control diet in the Bruce et al. (2006) study. Therefore, this difference in initial soybean meal composition may be affecting the overall digestibility and the pigs' response to soybean by-products.

Besides the improvement in ADG reported during the final phase of the nursery period, there were no other differences in growth performance. Therefore, although there is potential for these by-products to be added back to soybean meal, it will likely have limited effects on swine performance in the nursery phase due to the low inclusion levels in soybean meal. Perhaps in early finishing pig diets (25 to 90 kg), where SBM levels are greater than in the nursery phase, there might be greater potential to see improvement in ADG and G:F. Anecdotal reports suggest swine producers have noted concerns of increased diarrhea and scouring when sourcing soybean meal from specific locations or plants. Based on the results of this study, these observations are likely not linked to the inclusion of by-products back to soybean meal. This is further demonstrated by the lack of difference in fecal DM percentage noted when pigs were fed soybean meal containing by-products compared to soybean meal devoid of soybean gums and soapstocks.

In summary, these data suggest that adding soybean processing by-products such as soybean gums or soybean soapstocks had minimal effects on nursery pig growth performance.

However, there is a potential for improved ADG and ATTD of DM in the late nursery period when soybean meal containing added gums are included in the diet. This also coincides with when diets contained the greatest amount of soybean meal and therefore the greatest amounts of gums and soapstocks. Swine producers can source soybean meal without concerns of added soybean by-products negatively effecting nursery pig health or performance. Further research is needed to understand how soybean by-products would affect growth performance of pigs when added at higher inclusion levels independent of soybean meal or at different phases of growth.

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Table 6.1. Diet composition (as-fed basis)[1]

Item	Phase 1			Phase 2			Phase 3		
	Treatment 1	Treatments 2 – 4	Treatment 5	Treatment 1	Treatments 2 - 4	Treatment 5	Treatment 1	Treatments 2 - 4	Treatment 5
Ingredient, %									
Corn	50.05	49.96	50.72	55.71	55.66	56.78	65.55	65.40	66.64
Soybean meal[2]	18.61	18.61	17.85	27.95	27.95	26.76	30.54	30.54	29.34
Spray-dried whey	20.00	20.00	20.00	10.00	10.00	10.00	---	---	---
Enzymatically treated soybean meal[3]	3.75	3.75	3.75	---	---	---	---	---	---
Fish meal	4.50	4.50	4.50	2.00	2.00	2.00	---	---	---
Monocalcium P	0.60	0.60	0.60	1.05	1.05	1.05	0.95	0.95	0.95
Calcium carbonate	0.30	0.30	0.30	0.53	0.53	0.53	0.85	0.85	0.85
Zinc oxide	0.40	0.40	0.40	0.27	0.27	0.27	---	---	---
Salt	0.40	0.40	0.40	0.50	0.50	0.50	0.60	0.60	0.60
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Lys-HCl	0.39	0.42	0.42	0.44	0.48	0.48	0.48	0.52	0.52
DL-Met	0.20	0.21	0.21	0.22	0.23	0.23	0.20	0.21	0.21
L-Thr	0.18	0.19	0.19	0.20	0.22	0.22	0.22	0.23	0.23
L-Trp	0.05	0.05	0.05	0.04	0.05	0.05	0.04	0.05	0.05
L-Val	0.11	0.12	0.12	0.13	0.15	0.15	0.12	0.14	0.14
L-Ile	---	0.03	0.03	---	0.03	0.03	---	0.02	0.02
Phytase[4]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Titanium dioxide[5]	---	---	---	0.50	0.50	0.50	---	---	---
Total	100	100	100	100	100	100	100	100	100
Calculated analysis[6]									
SID AA, %									
Lys, %	1.35	1.35	1.35	1.35	1.35	1.35	1.30	1.30	1.30
Ile:Lys	58	58	58	57	57	57	56	56	56

Leu:Lys	114	112	113	114	111	112	117	114	115
Met:Lys	38	38	38	38	38	38	37	37	37
Met and Cys:Lys	58	58	58	59	59	59	58	58	58
Thr:Lys	64	64	64	64	64	64	64	64	64
Trp:Lys	19.7	19.7	19.7	19.5	19.7	19.7	19.2	19.4	19.4
Val:Lys	70	70	70	70	70	70	70	70	70
His:Lys	34	33	33	35	34	34	37	36	36
NE, kcal/kg[7]	2,491	2,476	2,496	2,427	2,405	2,436	2,425	2,401	2,434
SID Lys:NE, g/Mcal	5.42	5.45	5.41	5.56	5.61	5.55	5.36	5.41	5.35
CP, %	21.0	20.7	20.8	21.2	20.7	20.8	20.8	20.3	20.4
Ca, %	0.70	0.70	0.70	0.72	0.72	0.72	0.69	0.69	0.69
STTD P, %	0.60	0.59	0.59	0.58	0.57	0.58	0.47	0.46	0.46

[1]Phase 1 diets were fed from weaning to d 11, phase 2 from d 11 to 23, and phase 3 from d 23 to 42.

[2]Treatment 1 served as a control diet containing soybean meal with no added soybean by-products. Treatments 2 through 4 contained soybean meal with 4% added by-products. This includes two diets containing gums or soapstocks added at 4% of the soybean meal level in the diet and a diet containing soybean meal with 2% added soybean gums and 2% added soybean soapstocks. Treatment 5 served as a negative control containing 4% less soybean meal with no added by-products to have equal soybean protein as diets with added gums or soapstocks.

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

[4]Ronozyme HiPhos 2700 GT, DSM Nutritional Products, Parsippany, NJ was included at 2,000 FTU/kg to provide an estimated release of 0.12% STTD P for all diets.

[5]Utilized as an indigestible marker for apparent total tract digestibility calculations.

[6]Calculated analysis is based off nutrient profiles for ingredients listed in the NRC (2012).

[7]Energy values of the soybean gums and soapstocks were unknown and therefore not included in the calculated energy values.

Table 6.2. Analyzed soybean meal and diet composition (as-fed basis)[1]

Item	Dry matter	Ether extract	Crude protein	Ash	Crude fiber	NDF	Ca	P
Soybean meal								
No by-products[2]	89.94	1.06	48.00	6.36	4.75	7.55	0.69	0.63
4% gums	88.01	4.00	46.05	6.08	4.35	7.30	0.62	0.68
4% soapstocks	85.75	1.39	45.40	6.08	4.85	7.10	0.65	0.61
2% gums + 2% soapstocks	86.98	2.50	46.55	6.03	5.25	5.95	0.56	0.64
Complete diets								
Phase 1								
No by-products	88.90	2.61	20.85	6.37	1.90	5.60	0.91	0.70
4% gums	88.60	2.91	20.10	6.64	1.90	4.90	0.87	0.72
4% soapstocks	88.51	2.36	20.35	6.30	1.85	5.05	0.86	0.73
2% gums + 2% soapstocks	88.39	2.63	19.65	6.34	1.75	4.45	0.91	0.73
4% less soybean meal	88.58	2.38	20.45	6.26	1.70	6.35	1.02	0.68
Phase 2								
No by-products	88.44	2.23	20.15	6.45	2.05	7.10	0.84	0.74
4% gums	88.04	3.11	20.15	6.44	2.45	6.35	0.97	0.82
4% soapstocks	87.69	3.20	19.60	6.34	2.15	7.80	0.88	0.76
2% gums + 2% soapstocks	87.78	3.18	21.15	6.32	2.65	6.90	0.98	0.75
4% less soybean meal	88.40	2.37	20.10	6.42	2.35	5.80	0.66	0.69
Phase 3								
No by-products	87.66	2.41	20.65	4.78	2.30	5.20	1.16	0.66
4% gums	87.46	3.26	18.65	4.64	2.65	5.40	1.09	0.63
4% soapstocks	86.77	2.41	19.20	4.71	2.60	4.60	1.06	0.61
2% gums + 2% soapstocks	87.14	2.85	20.20	4.70	2.45	6.15	0.89	0.63
4% less soybean meal	87.64	2.39	19.40	4.61	2.80	7.45	1.01	0.64

[1]All soybean meal and complete diet samples were submitted for duplicate analysis of Ca and P at the K-State Research and Extension Soil Testing Laboratory in Manhattan, KS. All samples were also submitted to Midwest Laboratories Inc., Omaha, NE for duplicate analysis of proximate composition and neutral detergent fiber.

[2]Initial soybean meal was analyzed for trypsin inhibitor activity by the Plant Genetic Resource Unit of the USDA-ARS. The soybean meal used throughout this trial had a trypsin inhibitor activity of 3.84 TIU/mg of seed powder.

Table 6.3. Analyzed soybean processing by-products (as-is)[1,2]

Item	Soybean gums	Soybean soapstocks
Moisture and volatile matter, %	20.32	44.26
Insoluble impurities, %	2.32	1.87
Unsaponifiable matter, %	0.58	0.35
P, %	0.953	0.156
Peroxide value, meq/kg	---	0.6
p-Anisidine value, %	3.6	---
Viscosity, cps	14,250	---
Fat by acid hydrolysis, %	52.29	6.53
SFA, %[3]		
C16:0	15.30	15.31
C17:0	0.13	---
C18:0	3.93	4.60
C20:0	0.20	---
C22:0	0.40	0.53
C24:0	0.27	0.53
Total SFA	20.32	21.33
MUFA, %[3]		
C16:1	0.17	---
C18:1[4]	12.81	22.12
C20:1[4]	0.15	---
Total MUFA	13.23	22.65
PUFA, %[3]		
C18:2[4]	58.00	48.94
C18:3[4]	8.29	7.26
Total PUFA	66.44	56.02
Free Fatty acids, %[3]	12.75	0.51

[1]All soybean gums and soapstocks utilized in the trial were sourced from a single batch of each by-product.

[2]Both soybean by-product samples were submitted to Eurofins Nutrition Analytical Center (Des Moines, IA) for lipid quality analysis. All analyses besides viscosity were run in quadruplet. Viscosity was analyzed in duplicate.

[3]Presented as a percentage of extracted lipid.

[4]Concentration includes isomers.

MUFA = monounsaturated fatty acids. PUFA = polyunsaturated fatty acids. SFA = saturated fatty acids.

Table 6.4. Effects of soybean gums and soapstocks added to soybean meal (SBM) on growth performance, fecal dry matter (DM), and apparent total tract digestibility (ATTD) of DM of nursery pigs[1]

		By-products, % of SBM					*P* =				
Item	Control	4% Gums	4% Soapstocks	2% Gums + 2% Soapstocks	4% Less SBM	SEM	Treatment	Gums × Soapstocks	Gums	Soapstocks	Control v. 4% less SBM
BW, kg											
d 0	5.3	5.3	5.3	5.3	5.3	0.02	1.000	0.873	0.986	0.975	0.933
d 11	7.4	7.3	7.2	7.3	7.2	0.12	0.588	0.472	0.758	0.246	0.204
d 23	13.1	13.0	12.8	12.9	12.7	0.21	0.725	0.623	0.918	0.353	0.196
d 42	24.4	24.7	24.3	24.9	23.9	0.38	0.382	0.817	0.220	0.958	0.378
d 0 to 11 (Phase 1)											
ADG, g	188	183	169	179	166	9.9	0.444	0.448	0.757	0.238	0.115
ADFI, g	211	213	197	206	192	10.4	0.531	0.770	0.582	0.315	0.199
G:F, g/kg	889	860	854	872	867	22.4	0.833	0.285	0.813	0.605	0.484
d 11 to 23 (Phase 2)											
ADG, g	486	478	475	473	470	13.5	0.932	0.833	0.685	0.540	0.402
ADFI, g	658	670	655	653	648	20.3	0.953	0.723	0.808	0.620	0.735
G:F, g/kg	739	715	727	726	729	11.1	0.659	0.295	0.255	0.933	0.506
d 23 to 42 (Phase 3)											
ADG, g	584	610	597	620	581	12.7	0.133	0.885	0.053	0.348	0.868
ADFI, g	879	911	900	927	886	22.2	0.531	0.911	0.174	0.394	0.818
G:F, g/kg	664	671	665	670	658	9.6	0.860	0.926	0.539	0.981	0.621
d 0 to 42 (Overall)											
ADG, g	454	463	452	464	441	9.0	0.352	0.878	0.255	0.939	0.297
ADFI, g	642	661	646	659	635	16.2	0.744	0.864	0.351	0.944	0.772
G:F, g/kg	708	702	702	705	697	7.5	0.867	0.552	0.849	0.799	0.287
Fecal DM, %[2]											
d 11	20.19	19.69	19.41	19.32	20.20	0.649	0.800	0.771	0.670	0.404	0.992
d 24	18.86	19.87	19.20	19.42	19.63	0.649	0.839	0.518	0.313	0.933	0.372
ATTD of DM, %[3]	76.22[b]	79.77[a]	76.35[b]	75.78[b]	78.50[ab]	0.901	0.004	0.019	0.085	0.027	0.069

[1]A total of 350 weanling pigs (DNA 241 × 600; initially 5.3 ± 0.02 kg) approximately 19 d of age were used in a 42-d experiment with 5 pigs per pen and 14 pens per treatment.

[2]Feces from three pigs from each pen were pooled, weighed, and dried to measure fecal dry matter. Treatment × day, $P = 0.775$; Treatment, $P = 0.862$; Day, $P = 0.375$.

[3]Both ground feed and fecal samples were dried in a 135°C forced air drying oven for 2 h to determine percentage DM of the samples used for the titanium assay. Titanium dioxide concentration in both dried feed and fecal samples were analyzed in duplicate.

DM = dry matter. ATTD = apparent total tract digestibility.