Effects of feed enzymes and botanical blends in nursery pig diets on growth performance, bone characteristics, nutrient digestibility and intestinal morphology

by

Ty Kim

B.S., Kansas State University, 2022

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2024

Approved by:

Co-Major Professor
Robert Goodband

Approved by:

Co-Major Professor
Joel DeRouchey

Copyright

Abstract

This thesis contains three chapters that includes: 1) developing a phytase release curve to quantify the release of phytate-bound phosphorous in swine diets, 2) determining the effect of a botanical-derived feed additive containing extracts of capsicum oleoresin, clove essential oil, and garlic extract in diets with or without pharmacological levels of Zn and Cu, and 3) determining the effect of compound enzymes in reduced energy and protein diets. Chapter 1 utilized 280 nursery pigs to determine the effect of increasing phytase on nursery pig growth performance, bone characteristics, and plasma inositol concentrations. Increasing phytase from 250 to 2,000 FTU/kg in phosphorus deficient diets improved nursery pig growth performance, bone characteristics, and plasma inositol concentrations. Using formulated phytase concentrations, equations were developed for growth performance, bone ash weight, percentage bone ash, and bone density to predict aP release up to 2,000 FTU/kg of Sunphase HT phytase for 10- to 22- kg pigs. Chapter 2 involved 340 pigs to determine the effect of a botanical-derived feed additive containing capsicum oleoresin, clove essential oil, and garlic extract (CCG) on growth performance and fecal dry matter in nursery pig diets with or without pharmacological levels of Zn and Cu. Feeding pharmacological levels of Zn and Cu resulted in increased ADG and ADFI but lower fecal DM on d 21. Feeding CCG numerically increased ADG and ADFI in pigs not fed pharmacological levels of Zn and Cu and numerically decreased ADG and ADFI in pigs fed pharmacological levels of Zn and Cu. Chapter 3 utilized 355 pigs to determine the effects of compound enzymes, consisting of carbohydrases and protease, on growth performance, gut health, and nutrient digestibility in reduced energy and protein nursery pig diets. Pigs fed high energy diets had improved growth performance, ATTD of DM, CP, and ADF, and AID of Arg, Asp, and Trp compared to pigs fed low energy diets. Enzyme inclusion had no impact on overall

growth performance but had a negative impact on duodenal morphology, ATTD of DM and CP, and AID of Met in low energy diets. In summary, these experiments provide data on aP release of Sunphase HT phytase, effect of a botanical-derived feed additive in nursery pig diets, and effect of compound enzymes in reduced energy and protein diets.

Table of Contents

List of Figures

List of Tables

Chapter 1 - Determining the phosphorus release curve for Sunphase HT phytase in nursery pig diets

Abstract

A total of 280 pigs (DNA 241 × 600, initially 10.4 ± 0.24 kg) were used in a 21-d study to determine the available P (aP) release curve for Sunphase HT phytase (Wuhan Sunhy Biology Co., Ltd., Wuhan, P.R. China) when fed diets with a high phytate concentration. On d 21 post-weaning, considered d 0 of the study, pigs were blocked by average pen body weight (BW) and randomly allotted to 1 of 7 dietary treatments with 5 pigs per pen and 8 pens per treatment. 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 with increasing (0.11, 0.19, and 0.27%) aP from monocalcium P, or 4 diets with increasing phytase (250, 500, 1,000, or 2,000 FTU/kg) added to the diet formulated to 0.11% aP. All diets were corn-soybean meal-canola meal-based and were formulated to contain 1.24% SID Lys, a 1.10:1 total Ca:P ratio, and a calculated 0.32% phytate P. Prior to the beginning of the study, all pigs were fed a diet containing 0.11% aP from d 18 to 21 post-weaning. At the conclusion of the study, 1 pig, closest to the mean weight of each pen, was euthanized, and the right fibula, 10^{th} rib, and metacarpal were collected to determine bone ash and density. After cleaning, bones were submerged in ultra-purified water under vacuum for 4 h and then weighed to calculate density (Archimedes principle). For bone ash, bones were processed using the non-defatted method. From day 0 to 21, increasing aP from monocalcium P increased (linear, $P \leq 0.014$) ADG, G:F, and final BW. Pigs fed increasing phytase had increased (linear, $P \leq 0.045$) ADG, final BW, and plasma inositol concentration as well as improved (quadratic, $P = 0.023$) G:F. For bone characteristics, pigs fed increasing aP from inorganic P had a linear improvement

($P \leq 0.019$) in fibula bone ash weight and percentage bone ash, rib bone ash weight and bone density, and all metacarpal bone properties, with a quadratic response ($P \leq 0.030$) for fibula bone density and rib percentage ash. Additionally, pigs fed increasing phytase had increased ($P < 0.05$) bone ash weight, percentage bone ash, and bone density in either a linear or quadratic fashion depending on the bone analyzed. The available P release curve generated for Sunphase HT phytase for percentage bone ash combining values from right fibula, 10th rib, and metacarpal is: aP release, % = (0.360 × FTU) ÷ (2,330.250 + FTU).

Key Words: bone ash, growth, nursery pigs, phosphorus release, phytase, plasma inositol

Introduction

Most swine diets consist of plant-based ingredients containing phytate, which is a major storage form of P in plant-based ingredients (Ravindran et al., 1994). However, phytate-bound P is largely unavailable for digestion and absorption by swine due to inherently low endogenous levels of the digestive enzyme, phytase (Humer et al., 2015). As a result, most swine diets are formulated with an exogenous microbial phytase, which makes plant-derived dietary P more available for utilization (Selle and Ravindran, 2008). In turn, phytase decreases the need for dietary inclusion of inorganic forms of P, which lowers feed costs, reduces antinutritional properties of phytate, and minimizes environmental impact by reducing P excretion.

Dietary phosphorus concentration has a large impact on the development of bones. As a result, bone ash weight and percentage bone ash are used as indicators of phytase efficacy (Gourley et al., 2018; Wensley et al., 2020; Becker et al., 2021). Similar to a study conducted by Gaffield et al. (2023), the current study used bone ash weight and percentage bone ash

measurements from multiple bones (fibula, 10th rib, and metacarpal) as indicators of whole-body mineralization. While more investigation is needed to understand the release of P using phytase as measured using different bones, it is hypothesized that an assessment of phytase release using multiple bones will generate predicted aP release values that are more accurate.

As the feed industry develops new or next generation phytase sources, an evaluation of their efficacy is needed to properly formulate swine diets. Sunphase HT (Wuhan Sunhy Biology Co. Ltd., P.R. China), is a bacterial derived 6-phytase that originates from *Escherichia coli* and is expressed by Pichia Pastoris yeast. Therefore, the objective of this study was to evaluate the effects of a new phytase source (Sunphase HT) on growth performance and bone properties of 10 to 22-kg nursery pigs fed diets with a high phytate concentration (0.32% phytate) and to develop an aP release curve. This is the first study using bone mineralization to provide a range of aP release values for Sunphase HT phytase in pigs.

Materials and Methods

The protocol used in this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee (4485.31). The phytase premix was analyzed to determine inclusion rate and was found to contain 11,158,000 FTU/kg. Additionally, monocalcium P and limestone were analyzed for Ca and P concentrations, and these values were used in diet formulation (Table 1). The Ca and P concentrations for corn, soybean meal and canola meal were based on historical analysis of these ingredients used in our previous phytase studies (Gaffield et al., 2023). All diets contained 7.5% canola meal, and due to its high phytate P concentration, resulted in a basal diet with a calculated 0.32% phytate P (Table 3; NRC, 2012). All diets were formulated to a 1.10:1 total Ca:P ratio and to contain 1.24% standardized ileal

digestible (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. Dietary treatments were derived from eight, 1-ton pallets of basal diet (Table 2). For each treatment diet, a subset of basal diet from each of the 8 batches along with treatment-specific ingredients including limestone, monocalcium phosphate, sand, and Sunphase HT phytase were mixed to produce the 7 final experimental diets (Table 3). During bagging, complete diet samples were collected from every fourth bag using a feed probe, pooled, and stored at -20°C.

Animals and Housing

The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Each pen (1.22 × 1.22 m) contained a 4-hole, dry self-feeder, and nipple waterer for ad libitum access to feed and water.

A total of 280 pigs (DNA 241 × 600) were weaned at approximately 21 d of age. At weaning, pigs were randomly allotted to pens and fed common corn-soybean meal-dried whey-based starter diets formulated to contain 1.40 and 1.35% SID lysine in phase 1 and 2, respectively. On d 18 post-weaning, all pigs were fed the diet containing 0.11% aP for a 3-d period. Then, on d 21 post-weaning, considered d 0 of the study, pigs were blocked by BW (initially 10.4 ± 0.24 kg) and randomly allotted to 1 of 7 dietary treatments. 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 aP (0.11, 0.19, and 0.27%) from monocalcium P (with limestone added to maintain a Ca:P ratio of 1.10:1), or 4 diets with increasing phytase (250, 500, 1,000, and 2,000 FTU/kg) added to the diet containing 0.11% aP.

Throughout the experiment, pig and feeder weights were recorded every 7 d to determine ADG, ADFI, and G:F. At the conclusion of the 21-d study, blood was collected from the jugular vein of 1 pig, closest to the mean weight of each pen, using an Ethylenediaminetetraacetic acid (EDTA) anti-coagulant blood collection tube to determine plasma inositol concentrations. Blood samples were centrifuged at 4°C at 1,500 × g for 15 min and plasma frozen for later analysis of plasma inositol (University of North Dakota). For development of an internal standard, retained serum samples (10 µL) from the laboratory were mixed with 30 µL of 75% methanol containing 100 ng of myo-inositol-1,2,3,4,5,6-d_6 (Medical Isotopes, Pelham, NH). After vortexing and centrifuging for 10 min at 2,000 × g, 10 µL of supernatant was injected into an Liquid chromatography-mass spectrometry (LC-MS) system for quantification.

After blood collection, the same pig in each pen was euthanized via penetrating captive bolt, and the right fibula, 10^{th} rib, and metacarpal were collected, individually placed in plastic bags with permanent identification, and stored at -20°C. For bone analyses, leftover extraneous soft tissue and cartilage caps were removed from each bone. For bone density, bones were submerged in ultra-purified water under vacuum for 4 h. Bones were then suspended in a vessel of water and weighed. The weights were then used to calculate bone density (Archimedes principle; Williams et al., 2023). For bone ash, bones were processed using the non-defatted method (Wensley et al., 2020). Each bone was dried at 105°C for 7 d in a drying oven and subsequently ashed at 600°C 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., 2020).

Chemical Analysis

Two samples of each diet were submitted for analysis of Ca at the KSU Soils Lab, Manhattan, KS (AOAC 985.01, 2006), and average values were calculated. One sample of each

diet was submitted for analysis in triplicate for P at Midwest Laboratories, Omaha, NE (AOAC 985.01, 2006). Additionally, one sample of each diet was submitted for complete phytase and phytic acid analysis (Eurofins Nutrition Analysis Center, Des Moines, IA) using the AOAC official method 2000.12 (AOAC, 2000) and the method outlined in Analytical Biochemistry Vol. 77:536-539 (1977) for the respective analyses.

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 intercept. The base model was fit using the GLIMMIX procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC). Linear and quadratic contrasts were constructed within increasing inorganic P or phytase treatments. Results were considered significant with *P*-values ≤ 0.05 and were considered marginally significant with *P*-values ≤ 0.10.

For each pen fed the inorganic P diets, marginal intake of aP per day was calculated according to the following equation: dietary aP% minus 0.11% (the aP in the basal diet) multiplied by ADFI. Using the marginal aP release as the predictor variable, a standard curve was developed for each of the response criteria. The equation for the standard curve was used to calculate aP release from each pen fed the different phytase dosages (250, 500, 1,000, and 2,000 FTU/kg) 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 using the GLIMMIX procedure. Formulated phytase levels were used to calculate all release values. Additionally, to evaluate the average aP release generated using data from all three bones, treatment and bone were added as

fixed effects, and block and pen were added as random intercepts with pen being included to account for the subsampling associated with measuring multiple bones per pig.

A model was fit to calculate release values using non-linear regression to generate estimated aP release curves based on G:F, bone ash weight, percentage bone ash, and bone density using the following functional form:

$$aP\ release, \% = \frac{a\ \times (FTU/kg)}{b + (FTU/kg)}$$

The model coefficient a is a horizontal asymptote indicating the maximum release of aP for each response, and the model parameter b represents the vertical asymptote. The model parameters were estimated using the nls function from the stat package in R (version 4.2.1 (2022-06-23); R Core Team, 2021)) using the RStudio environment (Version 2.22.12.0.353; RStudio Team, 2021).

Results

Analysis for Ca, P, and phytase activity of final diets were similar to diet formulation (Table 3). Phytase activity of complete diets increased across the phytase treatments with analyzed phytase concentrations of 220, 400, 960, and 1,700 FTU/kg for the four treatments compared with calculated values of 250, 500, 1,000, and 2,000 FTU/kg, respectively. Analyzed phytase concentrations were below the calculated target for the 500 and 2,000 FTU/kg treatments. However, calculated values of phytase activity were used to determine aP release values. Additionally, analysis of final diets for phytic acid was similar across treatments at 1.15%, which at 28% P (Selle and Ravindran, 2008), results in 0.32% phytate P similar to diet formulation (Table 2).

From d 0 to 21, increasing aP from monocalcium P increased final BW (linear, P = 0.014), ADG (linear, P = 0.009), and G:F (linear, P = 0.002; Table 4). Pigs fed increasing

phytase had increased ADG (linear, $P = 0.011$) and final BW (linear, $P = 0.007$). Furthermore, G:F increased quadratically ($P = 0.023$) in pigs fed increasing phytase.

For bone characteristics, increasing aP from monocalcium P resulted in a linear increase ($P \leq 0.019$) for fibula bone ash weight and percentage bone ash, 10^{th} rib bone ash weight and bone density, and all metacarpal bone properties (Table 4). For fibula bone density and 10^{th} rib percentage bone ash, increasing aP from monocalcium P resulted in a quadratic increase ($P \leq 0.030$).

Similarly, increasing phytase resulted in a linear increase ($P < 0.001$) for fibula bone ash weight and percentage bone ash, with fibula bone density showing a quadratic response ($P = 0.005$). For rib bone properties, increasing dietary phytase resulted in a linear increase ($P < 0.001$) in 10^{th} rib bone ash weight, with rib bone density exhibiting a quadratic increase ($P = 0.047$) and rib percentage bone ash exhibiting a tendency for a quadratic response ($P = 0.080$). Furthermore, pigs fed increasing phytase had a linear increase ($P < 0.001$) in metacarpal percentage bone ash, with metacarpal bone ash weight and bone density increasing in a quadratic fashion ($P \leq 0.037$). Finally, pigs fed increasing phytase had a linear increase ($P = 0.045$) in plasma inositol concentration (Table 4).

The calculated percentage aP release for Sunphase HT phytase followed the same trends as the means listed above with calculated percentage aP released varying depending on the growth performance, specific bone, and bone characteristic measured (Table 5).

For Sunphase HT, this study has provided an aP release curve for use in swine diets using data from nursery pigs weighing 10 to 21-kg at inclusion levels between 250 and 2,000 FTU/kg (Figure 1). The response criteria considered in this trial influenced the magnitude of aP release differently as FTU inclusion rates increased. An aP release curve was not developed for ADG

due to a negative release value at 250 FTU/kg and more erratic values with increasing phytase. However, the aP (%) release equation generated for Sunphase HT for G:F is:

$$G{:}\,F{:}\,aP\ release,\%\ = \frac{0.245\ \times (FTU/kg)}{175.989 + (FTU/kg)}$$

The release values for each response criteria averaged across the three bones (fibula, 10th rib, and metacarpal) may provide the most robust estimate of aP release. The aP release equations generated based on measures of bone mineralization are:

$$Bone\ ash\ weight{:}\ aP\ release,\%\ = \frac{0.344\ \times (FTU/kg)}{1{,}156.954 + (FTU/kg)}$$

$$Percentage\ bone\ ash{:}\ aP\ release,\%\ = \frac{0.360\ \times (FTU/kg)}{2{,}330.250 + (FTU/kg)}$$

$$Bone\ density{:}\ aP\ release,\%\ = \frac{0.260\ \times (FTU/kg)}{944.019 + (FTU/kg)}$$

Discussion

The use of phytase in swine diets has become a common practice due to its ability to reduce antinutritional effects and increase nutritional value of feed ingredients (Bedford and Schulze, 1998). Inclusion of phytase in swine diets has shown improvements in production and economics, while reducing the impact of the swine industry on the environment (Selle and Ravindran, 2008). Phytases can generally hydrolyze up to 60 to 70% of phytate in the diet, resulting in increased P available to the pig (Newkirk and Classen, 1998; Adeola and Cowieson, 2011). The diets in the study herein contained 1.15% phytic acid, and considering phytic acid is comprised of 28.2% P (Selle and Ravindran, 2008), this provided a calculated value of 0.32% phytate P in each diet.

The first microbial phytases were derived from the fungi *Aspergillus ficuum* in 1990 and commercialized in 1991 (Simons et al., 1990; Dersjant-Li et al., 2014). However, after the initial discovery, there was increased interest in improving the efficacy of phytase sources, as this can be affected by optimal pH, resistance to protease, and affinity to phytate (Dersjant-Li et al., 2014). This led to the discovery and development of *Escherichia coli* derived bacterial phytase. *Escherichia coli* derived phytases, like Sunphase HT, have been identified as more effective than those derived from fungi (Lei et al., 2013). Specifically, bacterial derived phytases have been shown to have a greater affinity to phytate and higher resistance to degradation by protease (Adeola and Cowieson, 2011).

Microbial phytase sources can also be classified as 3-phytase or 6-phytase, indicating the position at which hydrolysis of the phytate molecule begins (Selle and Ravindran, 2007). The phytase used in this study, Sunphase HT, is a 6-phytase that originates from *Escherichia coli* and is expressed in Pichia Pastoris yeast. To increase efficacy, this new generation of phytase was developed to be stable up to temperatures of 75 to 80°C and pH conditions ranging from 4.0 to 5.0.

Phytate is a major storage form of P in cereal grains. To ensure the aP release for Sunphase HT phytase was not limited by available substrate, this study utilized canola meal to increase phytate in the diets (Gaffield et al., 2023). The phytate-bound P concentration of 0.65% for canola meal compares to 0.38% phytate-bound P concentration for soybean meal (NRC, 2012). Newkirk and Classen (1998) observed that 70 to 80% of the phytate in canola meal can be hydrolyzed by phytase. Therefore, the diets used in the current study were formulated to contain 0.32% phytate-bound P. If diets similar to those used in the current study were formulated using corn and soybean meal, the phytate-bound P would be approximately 0.24%, which is lower than

diets containing canola meal. The higher dietary phytate inclusion was utilized to ensure that phytate substrate was not a limiting factor in estimating the phytase response.

Phytic acid is a hexaphosphoric acid ester of myo-inositol, or an inositol ring combined with six phosphate molecules, that can inhibit the availability of phosphorus and other minerals (McDowell, 2000). Lu et al. (2019) and Walk et al. (2013) hypothesized that extra-phosphoric effects observed with the use of phytase could be related to the release of myo-inositol and mitigation of anti-nutritional effects of phytate. This could be due to the insulin-like characteristics and function of myo-inositol in cellular processes (Croze and Soulage, 2013). Cowieson et al. (2013) observed improved growth performance with the supplementation of myo-inositol in broiler chickens. Studies conducted by Cowieson et al. (2017) and Lu et al. (2019) observed increased plasma inositol concentrations when phytase was included up to 3,000 FTU/kg in the diet. The current study observed increased plasma inositol concentrations when phytase was included up to 2,000 FTU/kg, which indicates an increase in the amount of phytate that was completely dephosphorylated.

Phosphorus is the second most abundant mineral in the body and is important for many biological functions (Berndt and Kumar, 2009). The P requirement for maximizing bone mineralization is greater than that to maximize growth performance (Vier et al., 2019). As a result, several studies have determined aP release for different response criteria (Wensley et al., 2020; Becker et al., 2021; Gaffield et al., 2023). Similarly, the current study determined different aP release values for several response criteria. Williams (2023) determined differences in bone mineralization across different bones in nursery pigs. The authors observed fibulas and 2nd ribs are more sensitive to differences in dietary P levels compared to metacarpal and 10th ribs in nursery pigs. However, in a similar study, Williams (2023) observed 10th ribs were the most

sensitive to detect dietary P differences in finishing pigs. As a result, the current study assessed bone properties using multiple bones (fibula, 10th rib, and metacarpal) to have a better understanding of total body mineralization. In the current study, the 10th rib, a non-weight bearing bone, exhibited the highest aP release when assessing bone density and percentage bone ash. However, the metacarpal was observed to have the lowest aP release for percentage bone ash, and the fibula was observed to have the lowest aP release for bone density. As a result of the variation across bones, the aP release for each response criterion averaged across the 3 bones may provide the best information regarding aP release.

When new or next generation phytase sources are developed and brought to market, an evaluation of their efficacy is needed. The current study observed an improvement in growth performance and bone characteristics in either a linear or quadratic fashion depending on the response. The greatest aP release was observed when Sunphase HT was included at 2,000 FTU/kg for all growth performance criteria as well as bone ash weight and percentage bone ash, indicating phytase levels above our highest inclusion rate tested may release even more aP.

This study has provided a range of aP release values for Sunphase HT phytase in nursery pigs weighing 10 to 21 kg when fed levels between 250 and 2,000 FTU/kg in diets with high phytate concentration. In summary, both growth performance and bone characteristics increased with increasing phytase in the diet. The aP release at different phytase inclusion levels varied depending on response criteria and specific bone. In general, a higher aP release was observed with growth performance compared to bone characteristics, which was not unexpected based on previous research (Wensley et al., 2020; Becker et al., 2021; Gaffield et al., 2023). Equations for aP release were developed for G:F and bone density and percentage bone ash of each of the three bones (fibula, 10th rib, and metacarpal) as well as the average of the three bones. The release

values determined using the average of the fibula, 10th rib, and metacarpal may provide the most robust estimate of aP release.

Acknowledgements

Appreciation is expressed to Wuhan Sunhy Biology Co. Ltd. (Wuhan, P.R. China) for partial financial support.

Literature Cited

Adeola, O., and A. J. Cowieson. 2011. BOARD-INVITED REVIEW: Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J. Anim. Sci. 89:3189–3218. doi:10.2527/jas.2010-3715

AOAC. 2000. Official methods of analysis of AOAC international. 17th ed. Gaithersburg, (MD): Association of Official Analytical Chemists.

AOAC. 2006. Official methods of analysis AOAC international. 18th ed. Arlington (VA): Association of Official Analytical Chemists.

Becker, L. L., M. R. Wensley, J. M. DeRouchey, J. C. Woodworth, M. D. Tokach, R. D. Goodband, J. T. Gebhardt, R. M. Raab, and P. A. Lessard. 2021. Determining the phosphorus release of GraINzyme phytase in diets for nursery pigs. Transl. Anim. Sci. 5:1–10. doi:10.1093/tas/txab105.

Bedford, M. R., and H. Schulze. 1998. Exogenous enzymes for pigs and poultry. Nutr. Res. Rev. 11:91–114. doi:10.1079/NRR19980007.

Berndt, T., and R. Kumar. 2009. Novel mechanisms in the regulation of phosphorus homeostasis. Physiology. 24:17–25. doi:10.1152/physiol.00034.2008.

Cowieson, A. J., A. Ptak, P. Maćkowiak, M. Sassek, E. Pruszyńska-Oszmałek, K. Żyła, S. Świątkiewicz, S. Kaczmarek, and D. Józefiak. 2013. The effect of microbial phytase and

myo-inositol on performance and blood biochemistry of broiler chickens fed wheat/corn-based diets. Poult. Sci. 92:2124–2134. doi:10.3382/ps.2013-03140.

Cowieson, A. J., F. F. Roos, J.-P. Ruckebusch, J. W. Wilson, P. Guggenbuhl, H. Lu, K. M. Ajuwon, and O. Adeola. 2017. Time-series responses of swine plasma metabolites to ingestion of diets containing *myo*- inositol or phytase. Br. J. Nutr. 118:897–905. doi:10.1017/S0007114517003026.

Croze, M. L., and C. O. Soulage. 2013. Potential role and therapeutic interests of myo-inositol in metabolic diseases. Biochimie. 95:1811–1827. doi:10.1016/j.biochi.2013.05.011.

Dersjant-Li, Y., A. Awati, H. Schulze, and G. Partridge. 2014. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. J. Sci. Food Agric. 95:878–896. doi:10.1002/jsfa.6998.

Gaffield, K. N., H. R. Williams, L. L. Becker, J. M. DeRouchey, J. C. Woodworth, M. D. Tokach, R. D. Goodband, J. T. Gebhardt, and J. M. Faser. 2023. Determining the phosphorus release curve for Smizyme TS G5 2,500 phytase from 500 to 2,500 FTU/kg in nursery pig diets. Transl. Anim. Sci. doi:10.1093/tas/txad090.

Gourley, K. M., J. C. Woodworth, J. M. DeRouchey, S. S. Dritz, M. D. Tokach, and R. D. Goodband. 2018. Determining the available phosphorus release of Natuphos E 5,000 G phytase for nursery pigs. J. Anim. Sci. 96:1101–1107. doi:10.1093/jas/sky006.

Humer, E., C. Schwarz, and K. Schedle. 2015. Phytate in pig and poultry nutrition. J. Anim. Physiol. Anim. Nutr. 99:605–625. doi:10.1111/jpn.12258.

Lei, Xin Gen, J. D. Weaver, E. Mullaney, A. H. Ullah, M. J. Azain. 2013. Phytase, a New Life for an “Old” Enzyme. Annu. Rev. Anim. Biosci. 1:283–309. doi:10.1146/annurev-animal-031412-103717.

Lu, H., A. J. Cowieson, J. W. Wilson, K. M. Ajuwon, and O. Adeola. 2019. Extra-phosphoric effects of super dosing phytase on growth performance of pigs is not solely due to release of myo-inositol. J. Anim. Sci. 97:3898–3906. doi:10.1093/jas/skz232.

McDowell, L. R. 2000. Vitamin-Like Structures. In: *Vitamins in Animal and Human Nutrition*, 2nd ed. doi:10.1002/9780470376911

Newkirk, R. W., and H. L. Classen. 1998. In vitro hydrolysis of phytate in canola meal with purified and crude sources of phytase. Anim. Feed Sci. Technol. 72:315–327. doi:10.1016/S0377-8401(97)00190-9.

NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington D.C.

Ravindran, V., G. Ravindran, and S. Sivalogan. 1994. Total and phytate phosphorus contents of various foods and feedstuffs of plant origin. Food Chem. 50:133–136. doi:10.1016/0308-8146(94)90109-0.

R Core Team, 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rstudio Team, 2021. Rstudio: Integrated Development for R. Rstudio, PBC, Boston, MA.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1–41. doi:10.1016/j.anifeedsci.2006.06.010.

Selle, P. H., and V. Ravindran. 2008. Phytate-degrading enzymes in pig nutrition. Livest. Sci. 113:99–122. doi:10.1016/j.livsci.2007.05.014.

Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. A. Kemme, P. Slump, K. D. Bos, M. G. E. Wolters, R. F. Beudeker, and G. J. Verschoor. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 64:525-540. doi:10.1079/BJN19900052.

Vier, C. M., S. S. Dritz, F. Wu, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, M. A. D. Gonçalves, U. A. D Orlando, K. Chitakasempornkul, and J. C. Woodworth. 2019. Standardized total tract digestible phosphorus requirement of 24- to 130-kg pigs. J. Anim. Sci. 97(10):4023-4031. doi:10.1093/jas/skz256.

Walk, C. L., M. R. Bedford, T. S. Santos, D. Paiva, J. R. Bradley, H. Wladecki, C. Honaker, and A. P. McElroy. 2013. Extra-phosphoric effects of superdoses of a novel microbial phytase. Poult. Sci. 92:719–725. doi:10.3382/ps.2012-02727.

Wensley, M. R., J. M. DeRouchey, J. C. Woodworth, M. D. Tokach, R. D. Goodband, S. S. Dritz, J. M. Faser, and B. L. Guo. 2020. Determining the phosphorus release of Smizyme TS G5 2,500 phytase in diets for nursery pigs. Transl. Anim. Sci. 4:1–9. doi:10.1093/tas/txaa058.

Williams, H. R. 2023. *The comparison of analytical methods used to measure bone mineralization across different bones, ages of pigs, dietary differences, and health statuses. (Ph.D. Dissertation).* Manhattan, KS: Kansas State University.

Williams, H. R., T. E. Chin, M. D. Tokach, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, J. R. Bergstrom, M. C. Rahe, C. L. Siepker, P. Sitthicharoenchai, S. L. Radke, S. M. Ensley, and J. T. Gebhardt. 2023. The effect of bone and analytical methods on the assessment of bone mineralization response to dietary phosphorus, phytase, and vitamin D in nursery pigs. J. Anim. Sci. skad353. doi:10.1093/jas/skad353.

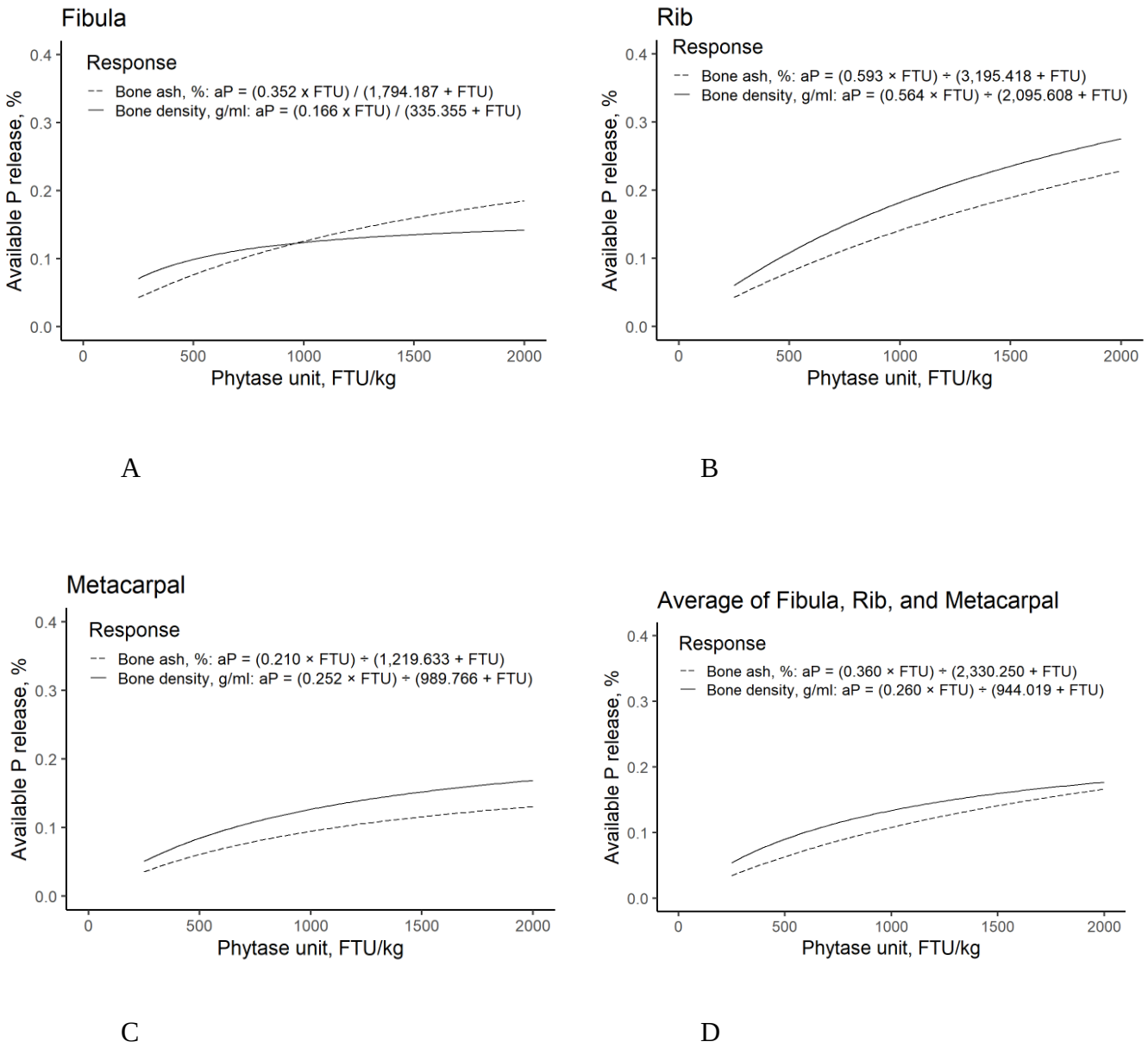

Figure 1.1. Available P release curves for A) right fibula, B) right rib, C) right metacarpal, and D) average of all three bones including percentage bone ash and bone density generated using the release equations for Sunphase HT from this experiment.

Table 1.1. Analyzed ingredient composition (as-fed basis)[1]

Ingredient	Ca, %	P, %
Limestone	37.75	0.01
Monocalcium P	18.57	23.09

[1]Ingredient samples were pooled and analysis was performed at the Kansas State University Soils Lab, Manhattan. Values represent the means of 3 samples analyzed in duplicate.

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

Item	
Ingredient, %	
Corn	60.94
Soybean meal	29.93
Canola meal	7.61
Sodium chloride	0.61
L-Lys-HCl	0.30
DL-Met	0.10
L-Thr	0.10
L-Val	0.01
Trace mineral premix	0.15
Vitamin premix	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	63
Trp:Lys	18.7
Val:Lys	71
His:Lys	43
NE, kcal/kg	2,414
SID Lys:NE, g/Mcal	5.14
CP, %	22.6
Ca, %	0.33
P, %	0.41
Available P, %	0.07
STTD P, %	0.17
Phytate P, %	0.32

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

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

	aP, %			Phytase, FTU/kg[2]			
Item	0.11	0.19	0.27	250	500	1,000	2,000
Ingredient, %							
Basal mix	98.62	98.62	98.62	98.62	98.62	98.62	98.62
Limestone	0.37	0.43	0.49	0.37	0.37	0.37	0.37
Monocalcium P	0.19	0.54	0.89	0.19	0.19	0.19	0.19
Sand[3]	0.82	0.41	0.00	0.82	0.81	0.81	0.80
Phytase[4]	----	----	----	0.0022	0.0045	0.0090	0.0179
Total	100	100	100	100	100	100	100
Calculated analysis							
CP, %	22.3	22.3	22.3	22.3	22.3	22.3	22.3
Ca, %	0.50	0.59	0.67	0.50	0.50	0.50	0.50
P, %	0.45	0.53	0.61	0.45	0.45	0.45	0.45
Phytase, FTU/kg	----	----	----	250	500	1,000	2,000
Ca:P ratio	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Phytate P, %	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Analyzed composition[5]							
Ca, %	0.51	0.57	0.76	0.50	0.47	0.53	0.53
P, %	0.48	0.54	0.65	0.48	0.47	0.47	0.49
Phytase, FTU/kg[6]	----	----	----	220	400	960	1,700
Phytic acid, %[6]	1.15	1.16	1.18	1.12	1.17	1.12	1.17

[1]Diets were fed for 21 d starting at approximately 10.4 ± 0.24 kg BW.

[2]Sunphase HT, Wuhan Sunhy Biology Co., Ltd., Wuhan, P.R. China.

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

[4]Phytase was analyzed and contained 11,158,000 FTU/kg (Wuhan Sunhy Biology Co., Ltd., Wuhan, P.R. China).

[5]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 analysis of Ca (Kansas State University Soils Lab, Manhattan) and P (Midwest Laboratories, Omaha, NE) using the AOAC official method 985.01 (AOAC, 2006).

[6]One sample of each diet was submitted to Eurofins Nutrition Analysis Center (Des Moines, IA) for complete phytase and phytic acid analysis using the AOAC official method 2000.12 (AOAC, 2000) and the method outlined in Analytical Biochemistry Vol. 77:536-539 (1977) for the respective analyses.

Table 1.4. Effects of increasing aP from monocalcium P or Sunphase HT phytase on nursery pig growth performance and bone ash values[1,2]

	aP, %[3]			Phytase, FTU/kg[4]					aP, *P* =		Phytase, *P* =	
Item	0.11	0.19	0.27	250	500	1,000	2,000	SEM	Linear	Quadratic	Linear	Quadratic
BW, kg												
d 0	10.4	10.5	10.4	10.4	10.4	10.4	10.4	0.24	0.440	0.566	0.941	0.696
d 21	20.7	21.3	22.0	20.8	21.6	21.5	22.1	0.44	0.014	0.877	0.007	0.487
d 0 to 21												
ADG, g	490	510	552	482	526	505	545	16.7	0.009	0.589	0.011	0.987
ADFI, g	772	776	818	724	773	743	791	23.5	0.143	0.463	0.250	0.258
G:F, g/kg	634	658	676	666	680	679	689	10.1	0.002	0.820	0.001	0.023
Bone characteristics[5]												
Fibula												
Bone ash, g	0.600	0.734	0.821	0.733	0.707	0.844	0.956	0.038	< 0.001	0.615	< 0.001	0.231
Bone ash, %	43.1	46.2	47.8	44.5	44.9	47.3	48.3	0.848	< 0.001	0.468	< 0.001	0.158
Bone density, g/mL	1.15	1.22	1.23	1.19	1.20	1.22	1.23	0.012	< 0.001	0.030	< 0.001	0.005
Rib												
Bone ash, g	0.753	0.913	1.168	0.945	0.922	1.102	1.318	0.061	< 0.001	0.530	< 0.001	0.408
Bone ash, %	46.4	50.8	51.0	48.5	48.9	51.2	53.1	0.692	< 0.001	0.016	< 0.001	0.080
Bone density, g/mL	1.20	1.22	1.24	1.20	1.22	1.26	1.25	0.011	0.019	0.932	< 0.001	0.047
Metacarpal												
Bone ash, g	0.872	1.073	1.277	1.053	1.160	1.182	1.359	0.039	< 0.001	0.972	< 0.001	0.018
Bone ash, %	32.5	34.7	37.7	32.5	34.5	35.7	36.1	0.828	< 0.001	0.723	< 0.001	0.119
Bone density, g/mL	1.13	1.16	1.18	1.15	1.16	1.17	1.18	0.006	< 0.001	0.338	< 0.001	0.037
Plasma inositol ng/μL	9.59	7.47	8.36	9.82	9.16	11.19	12.22	1.153	0.447	0.281	0.045	0.881

[1]A total of 280 nursery pigs (DNA 241 × 600, initially 10.4 ± 0.24 kg) were used in a 21-d growth trial with 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]Sunphase HT, Wuhan Sunhy Biology Co., Ltd., Wuhan, P.R. China.

[5]One pig per pen (8 pens per treatment) was euthanized and the right fibula, tenth rib, and metacarpal were collected to determine bone density, bone ash weight, 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 105ºC for 7 d and then ashed in a muffle furnace at 600ºC for 24 h.

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

	Phytase, FTU/kg[2]					*P* =	
Item	250	500	1,000	2,000	SEM[4]	Linear	Quadratic
Performance							
ADG	-0.017	0.102	0.037	0.146	0.0433	0.017	0.945
G:F	0.139	0.195	0.194	0.231	0.0455	0.001	0.018
Bone characteristics[3]							
Fibula							
Bone ash, g	0.098	0.073	0.181	0.252	0.0263	< 0.001	0.140
Bone ash, %	0.040	0.053	0.155	0.176	0.0318	< 0.001	0.105
Bone density, g/mL	0.079	0.082	0.139	0.137	0.0245	< 0.001	0.003
Rib							
Bone ash, g	0.086	0.073	0.147	0.228	0.0202	< 0.001	0.272
Bone ash, %	0.047	0.062	0.156	0.225	0.0211	< 0.001	0.032
Bone density, g/mL	-0.032	0.074	0.290	0.238	0.0529	< 0.001	0.027
Metacarpal							
Bone ash, g	0.078	0.119	0.135	0.197	0.0170	< 0.001	0.012
Bone ash, %	0.006	0.067	0.117	0.120	0.0271	0.001	0.081
Bone density, g/mL	0.043	0.082	0.139	0.163	0.0262	< 0.001	0.031
Average[4]							
Bone ash, g	0.085	0.091	0.150	0.223	0.0204	< 0.001	0.076
Bone ash, %	0.020	0.054	0.130	0.159	0.0528	0.007	0.308
Bone density, g/mL	0.045	0.080	0.171	0.166	0.0268	< 0.001	0.004

[1]The marginal intake of available P (aP) per day was calculated for each pen using the equation: dietary aP% minus 0.11% (the aP in the basal diet) multiplied by average daily feed intake. 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] Sunphase HT, Wuhan Sunhy Biology Co., Ltd., Wuhan, P.R. China.

[3] 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, 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^{o}C for 7 d and then ashed in a muffle furnace at 600^{o}C for 24 h.

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

Chapter 2 - Effects of a botanical feed additive blend of capsicum oleoresin, clove essential oil, and garlic extract on growth performance and fecal dry matter in nursery pigs

Abstract

A total of 340 barrows (DNA 200 × 400, initially 6.1 ± 0.08 kg BW) were used in a 38-d growth study to determine the effect of a botanical-derived feed additive containing extracts of capsicum oleoresin, clove essential oil, and garlic extract (CCG; Fytera Start, Selko, Indianapolis, IN) in diets with or without pharmacological levels of Zn and Cu on growth performance and fecal DM of nursery pigs. Pigs were weaned at 21 d of age, randomly allotted to pens based on initial BW, and pens were allotted to 1 of 4 dietary treatments in a completely randomized design. There were 5 pigs per pen and 17 pens per treatment. Diets were fed in three phases from d 0 to 10, 10 to 21, and 21 to 38, and treatments were arranged in a 2 × 2 factorial with main effects of CCG (none or 100 mg/kg) and nutritional or pharmacological levels of Zn and Cu. All diets contained 110 mg/kg of Zn and 16.5 mg/kg of Cu from the trace mineral premix, and these were the levels fed in the control diets. Pharmacological levels of Zn were added at 3,000 and 2,000 mg/kg in phase 1 and 2, respectively and Cu was added at 250 mg/kg in all phases. From d 0 to 21 and d 0 to 38, there was a CCG × Zn/Cu interaction observed for ADG and ADFI ($P < 0.05$) where CCG numerically increased ADG and ADFI in pigs not fed pharmacological levels of Zn/Cu; but reduced ADG and ADFI when pigs were fed pharmacological levels of Zn/Cu. Additionally, from d 0 to 38, there was a tendency for a CCG × Zn/Cu interaction observed for G:F ($P = 0.083$) in which the addition of CCG had no effect on G:F when pigs were fed nutritional levels of Zn and Cu, but the addition of CCG numerically decreased G:F in pigs fed pharmacological

levels of Zn and Cu. There was a Zn/Cu × day interaction ($P = 0.001$) for fecal dry matter, in which there was no difference ($P > 0.10$) in fecal DM on d 10, but pigs fed pharmacological levels of Zn/Cu had lower ($P < 0.001$) fecal DM on d 21 compared to pigs fed nutritional levels of Zn/Cu. Fecal DM was increased ($P < 0.001$) on d 10 compared to d 21. In summary, feeding pharmacological levels of Zn and Cu resulted in increased ADG and ADFI. Feeding CCG numerically increased ADG and ADFI in pigs fed nutritional levels of Zn and Cu and numerically decreased ADG and ADFI in pigs fed pharmacological levels of Zn and Cu. The response to CCG and pharmacological Zn/Cu were not additive.

Key Words: botanical extracts, copper, fecal dry matter, growth, nursery pigs, zinc

Introduction

Weaning is a stressful event in a pig's life and is often marked by poor performance and increased incidence of post-weaning diarrhea (Pluske et al., 1997). Adding pharmacological levels of Zn and Cu in nursery pig diets is a common strategy to improve growth performance and fecal consistency of nursery pigs (Hill et al., 2000; Bikker et al., 2016). The effects of pharmacological levels of Zn and Cu are well established and appear to be a result of their effect on gut morphology and antimicrobial activity (Højberg et al., 2005; Liu et al., 2018). However, with increasing pressure to reduce fecal mineral excretion and prevent bacterial resistance, there is renewed attention to find alternative feed additives that can elicit similar benefits.

Phytogenics are plant-derived feed additives that are hypothesized to generate similar results to pharmacological levels of Zn and Cu on nursery pig performance due to their effect on gut health as well as their antimicrobial and antioxidative activity (Franz et al., 2010). However,

due to differences in the composition of phytogenics, their effects on pig performance can vary (Franz et al., 2010). In literature reviews, Franz et al. (2010) and Zeng et al. (2015) summarized the results of numerous studies using capsicum oleoresin, clove, and garlic compounds (either independently or in combination with other plant compounds) and reported equivocal effects on growth performance. Zeng et al. (2015) reported three studies with capsicum oleoresin in combination with other compounds, with two observing positive effects and one observing negative effects on growth performance. Zeng et al. (2015) also reported three studies with clove in combination with other compounds, and all observed a positive response on growth performance. Recently a blend of botanical-derived active molecules comprised of capsicum oleoresin, clove essential oil, and garlic extract (CCG; Fytera Start, Selko, Indianapolis, IN) has been introduced for use in nursery pig diets. Previous research has shown decreased frequency of diarrhea and improved intestinal morphology when feeding CCG in pigs challenged with *E. coli* (Wong et al., 2022). However, no data is available to determining the effects of this CCG blend in diets with pharmacological levels of Zn and Cu. Our hypothesis was that this combination of botanically derived compounds would offer similar growth performance as pigs fed pharmacological levels of Zn and Cu, and their response might be additive. Therefore, the objective of this study was to evaluate the effects of CCG on growth performance and fecal DM of nursery pigs in diets with nutritional or pharmacological levels of Zn and Cu.

Materials and Methods

The protocol used in this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee (4506.12). The study was conducted at the Kansas State University Segregated Early Weaning facility in Manhattan, KS. The facility has two identical barns that are completely enclosed, environmentally controlled, and mechanically

ventilated. Each pen (1.22 × 1.22 m) had metal tri-bar floors and contained a 4-hole, dry self-feeder and a cup waterer for ad libitum access to feed and water.

A total of 340 barrows (DNA 200 × 400, initially 6.1 ± 0.08 kg BW) were used in a 38-d growth study. Pigs were weaned at approximately 21 d of age, randomly allotted to pens based on initial BW, and then pens were allotted to 1 of 4 dietary treatments in a completely randomized design. There were 5 pigs per pen and 17 pens per treatment across two barns (8 replicate pens in one barn and 9 replicate pens in the other).

Treatments were arranged in a 2 × 2 factorial with main effects of CCG (none or 100 mg/kg) and nutritional or pharmacological levels of Zn and Cu. All diets contained 110 mg/kg of Zn and 16.5 mg/kg of Cu from the trace mineral premix as the basal levels. Nutritional levels were 110 mg/kg of Zn and 16.5 mg/kg of Cu in all three phases. Pharmacological levels were 3,000 and 2,000 mg/kg of Zn in phase 1 and 2, respectively, and 250 mg/kg of Cu in all three phases. To achieve expected levels of Zn and Cu in the diet, Zn from ZnO and Cu from $CuSO_4$ were added. The experimental diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS (Table 1). Phase 1 diets were fed in pellet form, and phase 2 and 3 diets were fed in meal form. Complete diet samples were taken during bagging of experimental diets from every fourth bag using a feed probe, pooled into one homogenized sample per dietary treatment, and stored at -20°C until analysis. Complete diet samples were ground to reduce particle size and submitted for Zn and Cu analysis (Method 985.01; AOAC International, 2000; Cumberland Valley Analytical Services, Waynesboro, PA).

Individual pigs were weighed and feed disappearance was recorded on d 0, 10, 17, 21, 31, and 38 to determine ADG, ADFI, and G:F. Fecal samples were collected from the same three randomly selected pigs in each pen on d 10 and 21 of the experiment for fecal scoring and fecal

DM analysis. Immediately after collection, the same person assigned a score of 0, 1, or 2 to each sample with 0 = hard, 1 = soft, and 2 = diarrhea. Fecal scores of each sample were maintained separately for each pig and all 3 observations per pen were used for statistical analysis to determine the frequency of observations within each fecal score category. After collection, fecal samples were dried at 55°C in a forced air oven for 48 h, and the ratio of dried to wet fecal weight determined the fecal DM. Fecal samples were maintained separately for each pig and the average of the three samples from each pen was then used for statistical analysis.

Statistical Analysis

Growth performance data were analyzed as a completely randomized design with pen serving as the experimental unit. Bodyweight at d 0 was used as a covariate for all responses except d 0 BW. The main effects of CCG and Zn/Cu and their interactions were tested. Fecal DM data were analyzed as a completely randomized design with pen as the experimental unit with the fixed effects of day, treatment, and the associated interaction accounting for repeated measures over time. For fecal score analysis, data were analyzed as ordinal outcomes using a generalized linear mixed model using a multinomial response distribution using a cumulative logit link function. Pen was included in the model as a random intercept to account for multiple fecal score observations for each pen on each day. Data were summarized using the FREQ procedure of SAS and reported as percentage of observations within each fecal score category by CCG, Zn/Cu, and day. Growth and fecal DM data were analyzed using the lmer package of R (version 4.2.2 (2022-10-31)). Fecal score data were analyzed using the FREQ and GLIMMIX procedures of SAS (version 9.4; Cary, NC). Differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results

Analyzed Zn and Cu levels for all three phases were similar to the calculated values used in formulation (Table 2).

From d 0 to 10, there were no CCG × Zn/Cu interactions observed nor main effect of CCG (Table 3). However, pigs fed pharmacological levels of Zn and Cu had increased ADG, ADFI, G:F, and d 10 BW ($P < 0.001$) compared to pigs fed nutritional levels of Zn and Cu. From d 10 to 21 and d 0 to 21, there was a CCG × Zn/Cu interaction observed ($P < 0.05$) for ADG, ADFI, and d 21 BW where the addition of CCG resulted in a numerical increase in ADG and ADFI in pigs fed nutritional levels of Zn and Cu; however, in pigs fed pharmacological levels of Zn and Cu, the addition of CCG resulted in a numerical reduction in ADG and ADFI. Additionally, from d 10 to 21 pharmacological levels of Zn and Cu increased G:F ($P < 0.05$) compared to pigs fed nutritional levels of Zn and Cu.

From d 21 to 38, there was a CCG × Zn/Cu interaction observed ($P < 0.05$) for ADG and d 38 BW in which the addition of CCG resulted in a numerical increase in ADG in pigs fed nutritional levels of Zn and Cu; however, in pigs fed pharmacological levels of Zn and Cu, the addition of CCG resulted in a numerical reduction in ADG. Average daily feed intake was increased ($P < 0.05$) in pigs fed pharmacological additions of Zn and Cu compared to those fed nutritional levels. There was a tendency for a CCG × Zn/Cu interaction for G:F ($P = 0.064$) in which the addition of CCG numerically increased G:F in pigs fed nutritional levels of Zn and Cu, but the addition of CCG numerically decreased G:F in pigs fed pharmacological levels of Zn and Cu.

From d 0 to 38 (overall), there was a CCG × Zn/Cu interaction observed ($P < 0.05$) for ADG and ADFI in which the addition of CCG numerically increased ADG and ADFI in pigs fed

nutritional levels of Zn and Cu; however, when pigs were fed pharmacological levels of Zn and Cu, the addition of CCG numerically decreased ADG and ADFI. There was a tendency for a CCG × Zn/Cu interaction for G:F ($P = 0.083$) in which the addition of CCG had no effect on G:F when pigs were fed nutritional levels of Zn and Cu, but the addition of CCG numerically decreased G:F in pigs fed pharmacological levels of Zn and Cu.

For fecal dry matter, there was a Zn/Cu × day interaction observed ($P = 0.001$) in which there was no difference in fecal DM regardless of Zn and Cu level on d 10 ($P > 0.10$), but pigs fed pharmacological levels of Zn and Cu had lower fecal DM ($P < 0.001$) compared to those fed nutritional levels of Zn and Cu on d 21. There was also a tendency for a CCG × Zn/Cu interaction ($P = 0.075$) in which the addition of CCG numerically decreased fecal DM in pigs fed nutritional levels of Zn/Cu, but the addition of CCG numerically increased fecal DM in pigs fed pharmacological levels of Zn/Cu. Fecal DM was greater ($P < 0.001$) on d 10 compared to d 21. There was no diet effect on d 10 or 21 fecal scores but scores in general were lower (firmer feces; $P < 0.05$) on d 10 compared to d 21 which is consistent with the higher fecal DM on d 10 compared to d 21 (Figure 1).

Discussion

Zinc and copper are trace minerals required in growing pigs at 50 to 100 mg/kg and 3 to 6 mg/kg, respectively (NRC, 2012). However, including high levels, often referred to as pharmacological levels, of Zn from ZnO and Cu from $CuSO_4$ in nursery pig diets is a common practice in the U.S. to reduce post-weaning diarrhea and promote growth (Hill et al., 2000; Bikker et al., 2016). According to Liu et al. (2018), the positive effect of pharmacological levels of Zn on growth and fecal consistency is due to the mineral's effect on gut morphology and integrity and its anti-inflammatory activity. Højberg et al. (2005) suggested Zn also has anti-

microbial effects. Additionally, high levels of Cu can have the same impact due to its anti-microbial activity (Højberg et al., 2005; Liu et al., 2018).

The independent effects of pharmacological levels of Zn and Cu are well documented. Shelton et al. (2011) observed increased ADG, ADFI, and final BW in pigs fed 3,000 and 2,000 mg/kg Zn from ZnO in the first 21 to 28 days after weaning compared to pigs not fed pharmacological levels of Zn. Similarly, Pérez et al. (2011) observed increased overall nursery ADG and ADFI in pigs fed 250 mg/kg Cu from $CuSO_4$ compared to pigs not fed pharmacological levels of Cu. Hill et al. (2000) observed firmer fecal scores in pigs fed 3,000 mg/kg Zn from ZnO or 250 mg/kg Cu from $CuSO_4$ compared to pigs not fed pharmacological levels of Zn or Cu. Similar to Hill et al. (2000), in the current study, pigs fed pharmacological levels of Zn/Cu had increased ADG, ADFI, and BW throughout the overall nursery period compared to pigs not fed pharmacological levels of Zn and Cu.

Although the positive effects on growth performance and fecal DM are consistent, the use of pharmacological levels of Zn and Cu can have negative effects on the environment (Jondreville et al., 2003) and may lead to bacterial resistance (Yazdankhah et al., 2014). This has raised concern in some countries, leading to regulations to limit or prohibit the use of Zn and Cu as growth promoters. As a result, alternative feed additives that potentially elicit similar performance benefits need to be investigated.

Phytogenic compounds, or botanicals, are a relatively new class of feed additives with increasing interest since 2000, and they can include herbs, spices, essential oils, and oleoresins that are derived from plants (Windisch et al., 2008). The potential modes of action of plant derived feed additives include improved intestinal health, antimicrobial activity, antioxidative effects, and improved diet palatability (Franz et al., 2010). However, phytogenic feed additives

can vary based on plant and geographical origin, harvesting season, and processing technique (Windisch et al., 2008). These additives can be a single type of plant derived compound or a blend of compounds. As a result of the variation in composition, botanical feed additives can have a wide range of growth promoting, immunostimulatory, and antimicrobial benefits which can lead to inconsistent results (Turner et al., 2001).

The botanical additive used in the current study is a blend of extracts of capsicum oleoresin, clove essential oil, and garlic extract. Biggs et al. (2020) observed increased G:F with the addition of 0.01% capsicum oleoresin in diets without pharmacological levels of Zn/Cu when fed to pigs in thermoneutral and heat stress conditions. Long et al. (2020) observed increased ADG, ATTD of nutrients, and antioxidant capacity in pigs fed 1.6 mg/kg capsicum oleoresin compared to pigs fed 75 mg/kg CTC and pigs fed neither additive. Yan et al. (2011) observed increased ADG and ADFI in finishing pigs fed 0.2% garlic powder compared to pigs not fed garlic in diets without pharmacological levels of Zn/Cu. However, the authors did not observe further benefits when garlic powder was included at 0.4% of the diet. Huang et al. (2011) observed increased ADG and ADFI and improved feed efficiency in a linear or quadratic fashion as garlic inclusion increased from 0 to 0.025% in nursery pig diets with pharmacological levels of Cu. Mohammadi et al. (2014) observed increased ADG and improved feed efficiency in broiler chickens fed 300 mg/kg of clove essential oil compared to those not fed the essential oil. Liu et al. (2013) conducted a study to determine the effects of separately including 10 mg/kg of three different plant extracts (PE) including capsicum oleoresin, garlic botanical, or turmeric oleoresin in diets without pharmacological levels of Zn/Cu fed to pigs either infected or not infected with *Escherichia coli.* Although the authors did not include the same blend of botanical compounds, two of the compounds used were similar to the ones used in the current study

(capsicum oleoresin and garlic). Liu et al. (2013) observed increased ADG in the early nursery period in pigs fed either of the PE compared to the control when not infected with *E. coli.*, but there was no difference in overall performance. Similarly, pigs fed either of the PE had firmer fecal consistency compared to pigs fed the control regardless of health status. In a previous study by Wong et al. (2022), pigs fed 100 mg/kg of the same product used in the study herein in diets without pharmacological levels of Zn/Cu had numerically greater ADG and G:F and reduced frequency of diarrhea compared to pigs not fed the phytogenic additive when challenged with *E. coli* F18. There is little research on the interactive effects of phytogenics with other growth-promoting agents, such as pharmacological Zn and Cu. Feldpausch et al. (2018) observed a Cu × essential oil (EO) interaction where 125 mg/kg added Cu resulted in a numeric increase in G:F compared to pigs not fed high levels of Cu when EO was not included in the diet; however, when EO was included in the diet, pigs fed 125 mg/kg added Cu had a numeric reduction in G:F compared to pigs not fed high levels of Cu. In the current study, there was a CCG × Zn/Cu interaction on ADG and ADFI in which the addition of CCG numerically increased ADG and ADFI in pigs not fed pharmacological levels of Zn and Cu; however, in pigs fed pharmacological levels of Zn and Cu, the inclusion of CCG numerically decreased ADG and ADFI. The lack of additivity of botanical-derived feed additives with pharmacological levels of Zn/Cu could potentially be explained by similar effects of the two. Additionally, the benefits of pharmacological levels of Zn/Cu on nursery pig performance could reduce the potential for additional benefits from botanical-derived feed additives.

In summary, feeding pharmacological levels of Zn and Cu resulted in increased ADG and ADFI. A blend of extracts of capsicum oleoresin, clove essential oil, and garlic numerically increased ADG and ADFI in pigs fed nutritional levels of Zn and Cu and numerically decreased

ADG and ADFI in pigs fed pharmacological levels of Zn and Cu. There was no impact of the botanical blend or Zn and Cu level on fecal DM on d 10. However, feeding pharmacological levels of Zn and Cu resulted in lower fecal DM at d 21.

Acknowledgements

Appreciation is expressed to Selko USA (Indianapolis, IN) for partial financial support.

Literature Cited

AOAC. 2000. Official methods of analysis of AOAC international. 17th ed. Gaithersburg, (MD): Association of Official Analytical Chemists.

Biggs, M. E., K. A. Kroscher, L. D. Zhao, Z. Zhang, E. H. Wall, D. M. Bravo, and R. P. Rhoads. 2020. Dietary supplementation of artificial sweetener and capsicum oleoresin as a strategy to mitigate the negative consequences of heat stress on pig performance. J. Anim. Sci. 98:1-12. doi:10.1093/jas/skaa131.

Bikker, P., A. W. Jongbloed, and J. Van Baal. 2016. Dose-dependent effects of copper supplementation of nursery diets on growth performance and fecal consistency in weaned pigs1. J. Anim. Sci. 94:181–186. doi:10.2527/jas.2015-9874.

Feldpausch, J. A., R. G. Amachawadi, M. D. Tokach, H. M. Scott, S. S. Dritz, R. D. Goodband, J. C. Woodworth, and J. M. DeRouchey. 2018. Effects of dietary chlortetracycline, Origanum essential oil, and pharmacological Cu and Zn on growth performance of nursery pigs. Transl. Anim. Sci. 2:62–73. doi:10.1093/tas/txx004.

Franz, C., K. Baser, and W. Windisch. 2010. Essential oils and aromatic plants in animal feeding – a European perspective. A review. Flavour Fragr J. 25:327–340. doi:10.1002/ffj.1967.

Hill, G. M., G. L. Cromwell, T. D. Crenshaw, C. R. Dove, R. C. Ewan, D. A. Knabe, A. J. Lewis, G. W. Libal, D. C. Mahan, G. C. Shurson, L. L. Southern, and T. L. Veum. 2000.

Growth promotion effects and plasma changes from feeding high dietary concentrations of zinc and copper to weanling pigs (regional study). J. Anim. Sci. 78:1010. doi:10.2527/2000.7841010x.

Højberg, O., N. Canibe, H. D. Poulsen, M. S. Hedemann, and B. B. Jensen. 2005. Influence of dietary zinc oxide and copper sulfate on the gastrointestinal ecosystem in newly weaned piglets. Appl. Environ. Microbiol. 71:2267–2277. doi:10.1128/AEM.71.5.2267-2277.2005.

Huang, R. H., X. S. Qiu, F. X. Shi, C. L. Hughes, Z. F. Lu, and W. Y. Zhu. 2011. Effects of dietary allicin on health and growth performance of weanling piglets and reduction in attractiveness of faeces to flies. Animal. 5:304-311. doi:10.1017/S1751731110001953.

Jondreville, C., P. S. Revy, and J. Y. Dourmad. 2003. Dietary means to better control the environmental impact of copper and zinc by pigs from weaning to slaughter. Livest. Prod. Sci. 84:147–156. doi:10.1016/j.livprodsci.2003.09.011.

Liu, Y., M. Song, T. M. Che, J. A. S. Almeida, J. J. Lee, D. Bravo, C. W. Maddox, and J. E. Pettigrew. 2013. Dietary plant extracts alleviate diarrhea and alter immune responses of weaned pigs experimentally infected with a pathogenic *Escherichia coli*. J. Anim. Sci. 91:5294-5306. doi:10.2527/jas.2012-6194.

Liu, Y., C. D. Espinosa, J. J. Abelilla, G. A. Casas, L. V. Lagos, S. A. Lee, W. B. Kwon, J. K. Mathai, D. M. D. L. Navarro, N. W. Jaworski, and H. H. Stein. 2018. Non-antibiotic feed additives in diets for pigs: A review. Anim Nutr. 4:113–125. doi:10.1016/j.aninu.2018.01.007.

Long, S., S. Liu, J. Wang, S. Mahfuz, X. Piao. 2021. Natural capsicum extract replacing chlortetracycline enhances performance via improving digestive enzyme activities,

antioxidant capacity, anti-inflammatory function, and gut health in weaned pigs. Anim. Nutr. 7:305-314. doi:10.1016/j.aninu.2020.12.004.

Mohammadi, Z., S. Ghazanfari, and M. A. Moradi. 2014. Effect of supplementing clove essential oil to the diet on microflora population, intestinal morphology, blood parameters and performance of broilers. Eur. Poult. 78:1-11. doi:10.1399/eps.2014.51.

NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington D.C.

Pérez, V. G., A. M. Waguespack, T. D. Bidner, L. L. Southern, T. M. Fakler, T. L. Ward, M. Steidinger, and J. E. Pettigrew. 2011. Additivity of effects from dietary copper and zinc on growth performance and fecal microbiota of pigs after weaning. J. Anim. Sci. 89:414–425. doi:10.2527/jas.2010-2839.

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

R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Shelton, N. W., M. D. Tokach, J. L. Nelssen, R. D. Goodband, S. S. Dritz, J. M. DeRouchey, and G. M. Hill. 2011. Effects of copper sulfate, tri-basic copper chloride, and zinc oxide on weanling pig performance. J. Anim. Sci. 89:2440–2451. doi:10.2527/jas.2010-3432.

Turner, J. L., S. S. Dritz, and J. E. Minton. 2001. Review: alternatives to conventional antimicrobials in swine diets. Prof. Anim. Sci. 17:217-226. doi:10.15232/S1080-7446(15)31633-8.

Van Der Aar, P. J., F. Molist, and J. D. Van Der Klis. 2017. The central role of intestinal health on the effect of feed additives on feed intake in swine and poultry. Anim. Feed Sci. Technol. 233:64–75. doi:10.1016/j.anifeedsci.2016.07.019.

Verstegen, M. W. A., and B. A. Williams. 2002. Alternatives to the use of antibiotics as growth promoters for monogastric animals. Anim. Biotechnol. 13:113–127. doi:10.1081/ABIO-120005774.

Windisch, W., K. Schedle, C. Plitzner, and A. Kroismayr. 2008. Use of phytogenic products as feed additives for swine and poultry. J. Anim. Sci. 86:E140–E148. doi:10.2527/jas.2007-0459.

Wong, B. T., S. Park, L. Kovanda, Y. He, K. Kim, S. Xu, C. Lingga, M. Hejna, E. Wall, R. Sripathy, X. Li, and Y. Liu. 2022. Dietary supplementation of botanical blends enhanced performance and disease resistance of weaned pigs experimentally infected with enterotoxigenic *Escherichia coli* F18. J. Anim. Sci. 100:skac353. doi:10.1093/jas/skac353.

Yan, L., Q. W. Meng, X. Ao, T. X. Zhou, J. S. Yoo, H. J. Kim, and I. H. Kim. 2011. Effects of fermented garlic powder supplementation on growth performance, blood characteristics and meat quality in finishing pigs fed low-nutrient-density diets. Livest. Sci. 137:255-259. doi:10.1016/j.livsci.2010.09.024.

Yazdankhah, S., K. Rudi, and A. Bernhoft. 2014. Zinc and copper in animal feed – development of resistance and co-resistance to antimicrobial agents in bacteria of animal origin. Microb. Ecol. 25. doi:10.3402/mehd.v25.25862.

Zeng, Z., S. Zhang, H. Wang, and X. Piao. 2015. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. J. Anim. Sci. Biotechnol. 6:7. doi:10.1186/s40104-015-0004-5.

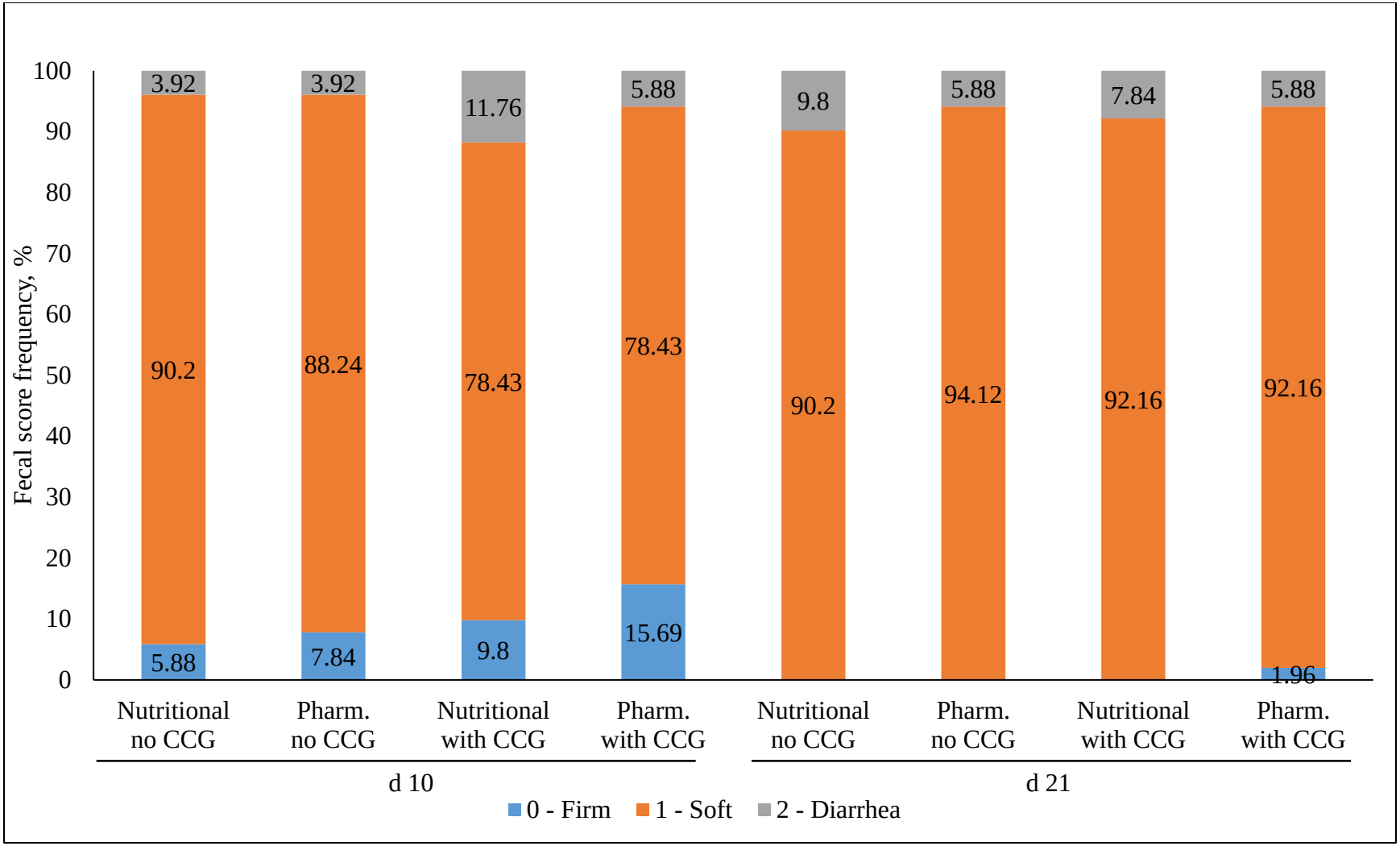

Figure 2.1. Interactive effect of capsicum oleoresin, clove essential oil, and garlic extract (Fytera Start, Selko, Indianapolis, IN), Zn/Cu dietary level, and day on fecal score frequency, P = 0.508. Main effect of Zn/Cu dietary level on fecal score frequency, *P* = 0.146. Main effect of day on fecal score frequency, *P* = 0.010. Nutritional levels of Zn/Cu were 110 and 16.5 mg/kg, respectively, for all three phases. Pharmacological levels of Zn were 3,000 and 2,000 mg/kg in phase 1 and 2, respectively, and pharmacological levels of Cu were 250 mg/kg in all three phases.

Table 2.1. Ingredient composition of experimental diets (as-fed basis)[1]

	Phase 1		Phase 2		Phase 3	
Ingredient, % Zn/Cu[2]:	Nutr.	Pharm.	Nutr.	Pharm.	Nutr.	Pharm.
Corn	45.29	44.76	57.57	57.18	65.75	65.65
Soybean meal	15.60	15.63	23.32	23.34	30.52	30.53
Fish meal	4.50	4.50	----	----	----	----
Whey powder	25.00	25.00	10.00	10.00	----	----
Enzymatically treated soybean meal[3]	5.00	5.00	5.00	5.00	----	----
Soybean oil	2.00	2.00	----	----	----	----
Limestone	0.25	0.25	0.83	0.83	0.81	0.81
Monocalcium P	0.65	0.65	1.18	1.18	0.83	0.83
Sodium chloride	0.30	0.30	0.55	0.55	0.60	0.60
L-Lys-HCl	0.40	0.40	0.50	0.50	0.48	0.48
DL-Met	0.21	0.21	0.22	0.22	0.20	0.20
L-Thr	0.18	0.18	0.22	0.22	0.21	0.21
L-Trp	0.04	0.04	0.04	0.04	0.04	0.04
L-Val	0.13	0.13	0.14	0.14	0.12	0.12
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix without phytase	0.25	0.25	0.25	0.25	0.25	0.25
Phytase[4]	0.06	0.06	0.06	0.06	0.06	0.06
Zinc oxide	----	0.40	----	0.26	----	----
Copper sulfate	----	0.09	----	0.09	----	0.09
CCG[5]	+/-	+/-	+/-	+/-	+/-	+/-
Total	100	100	100	100	100	100
Calculated analysis						
CP, %	20.5	20.4	20.7	20.7	20.8	20.8
Ca, %	0.71	0.71	0.77	0.77	0.65	0.65
STTD P, %	0.63	0.63	0.56	0.56	0.44	0.44
Ca:P	1.00	1.00	1.14	1.14	1.14	1.14
Zn, mg/kg	110	3,000	110	2,000	110	110
Cu, mg/kg	16.5	250	16.5	250	16.5	250

[1]Phase 1 diets were fed from d 0 to 10. Phase 2 diets were fed from d 10 to 21. Phase 3 diets were fed from d 21 to 38.

[2]Nutritional levels of Zn/Cu were 110 and 16.5 mg/kg, respectively, for all three phases. Pharmacological levels of Zn were 3,000 and 2,000 mg/kg in phase 1 and 2, respectively, and pharmacological levels of Cu were 250 mg/kg in all three phases.

[3]HP300; Hamlet Protein; Findlay, OH.

[4]Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) included at 1,486 FYT/kg provided an estimated release of 0.12% STTD P.

[5]Fytera Start (a blend of capsicum oleoresin, clove essential oil, and garlic extracts; Selko, Indianapolis, IN) was added at 0.01% to the diets with nutritional and pharmacological levels of Zn/Cu in each phase to create experimental treatments.

Table 2.2. Analyzed composition of experimental diets (as-fed basis)[1]

	Zn/Cu[2]:	Nutritional		Pharmacological	
Item, %	CCG[3]:	No	Yes	No	Yes
Phase 1					
Zn, mg/kg		149	154	3,103	2,597
Cu, mg/kg		23	22	250	258
Phase 2					
Zn, mg/kg		141	143	2,069	1,990
Cu, mg/kg		21	24	266	269
Phase 3					
Zn, mg/kg		139	164	160	128
Cu, mg/kg		26	33	253	228

[1]Complete diet samples were taken during bagging of experimental diets from every fourth bag, pooled into one homogenized sample per dietary treatment and analyzed for Zn and Cu (Method 985.01; AOAC International, 2000) at Cumberland Valley Analytical Services, Waynesboro, PA.

[2]Zinc from ZnO and copper from $CuSO_4$.

[3]Capsicum oleoresin, clove essential oil, and garlic extracts (Fytera Start; Selko, Indianapolis, IN).

Table 2.3. Interactive effect of a botanical blend and zinc and copper supplementation on nursery pig growth performance and fecal dry matter[1]

	Zn/Cu[2]:	Nutritional		Pharmacological			*P* =		
Item[3]	CCG[4]:	No	Yes	No	Yes	SEM	CCG × Zn/Cu	CCG	Zn/Cu
BW, kg									
d 0		6.1	6.1	6.0	6.1	0.08	0.545	0.008	0.608
d 10		6.8^{b}	6.9^{b}	7.3^{a}	7.2^{a}	0.16	0.242	0.932	< 0.001
d 21		11.1^{b}	11.6^{b}	13.0^{a}	12.5^{a}	0.26	0.007	0.886	< 0.001
d 38		21.2^{c}	22.1^{bc}	24.0^{a}	22.9^{ab}	0.35	0.002	0.811	< 0.001
d 0 to 10 (Phase 1)									
ADG, g		71^{b}	81^{b}	125^{a}	115^{a}	15.6	0.242	0.932	< 0.001
ADFI, g		107^{c}	112^{bc}	142^{a}	131^{ab}	15.9	0.184	0.630	< 0.001
G:F		0.64^{b}	0.70^{b}	0.86^{a}	0.86^{a}	0.044	0.438	0.556	< 0.001
d 10 to 21 (Phase 2)									
ADG, g		394^{b}	425^{b}	511^{a}	477^{a}	13.4	0.008	0.922	< 0.001
ADFI, g		463^{b}	506^{b}	624^{a}	582^{a}	17.0	0.006	0.993	< 0.001
G:F		0.85	0.84	0.82	0.82	0.013	0.725	0.645	0.033
d 0 to 21 (Phases 1 and 2)									
ADG, g		240^{b}	261^{b}	324^{a}	305^{a}	15.5	0.032	0.888	< 0.001
ADFI, g		293^{b}	318^{b}	391^{a}	367^{a}	15.5	0.019	0.942	< 0.001
G:F		0.82	0.82	0.83	0.83	0.012	0.861	0.937	0.248
d 21 to 38 (Phase 3)									
ADG, g		591^{b}	617^{ab}	645^{a}	615^{ab}	11.2	0.006	0.909	0.011
ADFI, g		829^{b}	860^{ab}	884^{a}	869^{ab}	16.2	0.114	0.632	0.029
G:F		0.71	0.72	0.73	0.71	0.008	0.064	0.351	0.600
d 0 to 38 (Overall)									
ADG, g		397^{c}	419^{bc}	467^{a}	443^{ab}	9.4	0.007	0.892	< 0.001
ADFI, g		533^{c}	559^{bc}	610^{a}	592^{ab}	13.6	0.048	0.780	< 0.001
G:F		0.75	0.75	0.77	0.75	0.007	0.083	0.450	0.087
Fecal DM[5], %									
d 10		25.6	23.8	25.1	25.5	0.93	0.154	0.323	0.398
d 21		24.1	23.8	20.5	21.7	0.93	0.271	0.578	< 0.001

[1]A total of 340 barrows (DNA 200 × 400, initially 6.1 ± 0.08 kg BW) were used in a 38-d growth trial with 5 pigs per pen and 17 replications per treatment.

[2]Zinc from ZnO was included in the diet at 110 or 3,000 mg/kg in phase 1, 110 or 2,000 mg/kg in phase 2, and 110 mg/kg in phase 3 and copper from $CuSO_4$ was included in the diet at 16.5 or 250 mg/kg throughout the 38-d study for the nutritional and pharmacological treatments respectively.

[3]BW d 0 was used as a covariate for all responses except BW d 0.

[4]Capsicum oleoresin, clove essential oil, and garlic extracts (Fytera Start; Selko, Indianapolis, IN) included in the diet at either 0 or 100 mg/kg throughout the 38-d study.

[5]CCG × Zn × Day, $P = 0.817$; CCG × Zn/Cu, $P = 0.075$; CCG × Day, $P = 0.275$; Zn/Cu × Day, $P = 0.001$; CCG, $P = 0.758$; Zn/Cu, $P = 0.026$; Day, $P < 0.001$.

Chapter 3 - Effects of compound enzymes in nursery pigs fed diets of different nutrient density

Abstract

A total of 355 nursery pigs (DNA 241 × 600, initially 13.3 ± 0.23 kg BW) were used in a 35-d growth study to determine the effects of a compound enzyme product containing xylanase, β-glucanase, β-mannanase, cellulase, pectinase, α-galactosidase, protease, and amylase on growth performance, intestinal morphology, and nutrient digestibility in nursery pigs fed diets of different nutrient density. At approximately 19 d of age, pigs were weaned, randomly allotted to pens, and fed common phase 1 and 2 diets. On d 24 post-weaning, considered d 0 of the study, pigs were blocked by average pen BW and allotted to 1 of 6 dietary treatments in a randomized complete block design with 4 or 5 pigs per pen and 12 pens per treatment. Treatment diets were formulated in two dietary phases and fed from d 0 to 22 and d 22 to 35, respectively. The six treatments included 2 corn-soybean meal-based diets (Corn-SBM; CS) with either 0 (CS + 0) or 0.01% (CS + 0.01) enzyme and 4 corn-soybean meal-wheat middling-low oil DDGS-based diets (Corn-SBM-By-product; CSBP) with lower dietary energy/CP with 0 (CSBP + 0), 0.01 (CSBP + 0.01), 0.02 (CSBP + 0.02), or 0.03% (CSBP + 0.03) enzyme. At the conclusion of the study, fecal samples were collected from 6 pigs per treatment to determine ATTD of DM, CP, ADF, and NDF. The same pigs were then euthanized, and ileal digesta and tissue samples were collected to determine AID of AA and small intestine morphology. Overall, pigs fed CS diets had increased ($P < 0.05$) ADG and G:F compared to pigs fed CSBP diets with no effects ($P > 0.05$) on ADFI. Pigs fed CSBP diets had increased ($P < 0.05$) duodenal villus height compared to pigs fed CS diets, but added enzyme decreased ($P = 0.017$) duodenal villus height in CS diets and decreased (linear, $P < 0.05$) duodenal villus height and VH:CD in CSBP diets. There were

no effects ($P > 0.05$) on jejunal or ileal morphology. Pigs fed CS diets had increased ($P < 0.05$) ATTD of DM, CP, and ADF, AID of Arg, Asp, and Trp compared to pigs fed CSBP diets. There was a quadratic reduction ($P = 0.015$) in AID of Met as enzyme increased in CSBP diets. In summary, pigs fed CS diets had improved growth performance, ATTD of DM, CP, and ADF, and AID of Arg, Asp, and Trp compared to pigs fed CSBP diets. The addition of a compound enzyme had no effect on overall growth performance but had a negative impact on duodenal morphology, ATTD of DM and CP, and AID of Met in CSBP diets.

Key Words: carbohydrase, digestibility, growth, intestinal morphology, nursery pigs, protease

Introduction

Swine diets typically include ingredients of plant origin, such as cereal grains, oilseeds, and their coproducts, which contain antinutritional factors (ANFs; Jacela et al., 2009; Woyengo, 2023). These components can include non-starch polysaccharides (NSPs) and protease inhibitors, which have negative effects on nutrient utilization and performance due to the pig's insufficient specific endogenous enzyme activity (Jha and Berrocoso, 2015). One strategy to reduce the negative effects of ANFs is supplementation of exogenous enzymes (Park and Adeola, 2023). Enzymes can be supplemented to swine diets to degrade feed components resistant to endogenous enzymes, inactive ANFs, and supplement endogenous enzymes that are present in insufficient amounts (Thacker, 2013).

Carbohydrases, specifically NSP-degrading enzymes, can act on the structural components of plant cell walls, that are otherwise indigestible by pigs, to increase nutrient availability (Masey O'Neill et al., 2014). Exogenous proteases, which supplement endogenous

protease produced by the pig, aid in the breakdown of protein molecules and act on protein based ANFs (Jacela et al., 2009). These enzymes can be included in the diet independently or in combination with other enzymes (Adeola and Cowieson, 2011). However, the use of exogenous enzymes in swine has generated variable results on growth performance and nutrient digestibility due to variability in enzyme source, diet composition, and physiological states of the animal (Park et al., 2016).

As a result, new enzyme products must be evaluated to determine their efficacy and potential use in swine diets. Sunzyme (Wuhan Sunhy Biology Co. Ltd., P.R. China) is comprised of NSP-degrading carbohydrases, such as xylanase (15,000 U/g), β-glucanase (2,500 U/g), β-mannanase (555 U/g), cellulase (300 U/g), pectinase (2,000 U/g), and α-galactosidase (100 U/g). In addition to NSP enzymes, it also contains digestive enzymes, such as protease (2,000 U/g) and amylase (3,000 U/g). There were two diet formulation strategies used in the current study, including corn-soybean meal diets and corn-soybean meal-by-product diets. The CSBP diets were formulated with a 3.2% reduction in NE and 1% reduction in crude protein (CP) compared to the CS diets. Our first hypothesis was that the addition of these compound enzymes in CSBP diets would result in similar growth performance, gut morphology, and nutrient digestibility as pigs fed CS diets. Our second hypothesis was that the addition of these compound enzymes in CS diets would improve growth performance, gut morphology, and nutrient digestibility. Therefore, the objective of this study was to determine the effects of compound enzymes on growth performance, intestinal morphology, and nutrient digestibility in nursery pigs fed diets of different nutrient density.

Materials and Methods

The protocol used in this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee (4485.44). 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 waterer for ad libitum access to feed and water.

A total of 355 nursery pigs (DNA 241 × 600) were weaned at approximately 19 d of age, randomly allotted to pens, and fed common phase 1 and 2 diets. On d 24 post-weaning, considered d 0 of the study, pigs were blocked by average pen BW (initially 13.3 ± 0.23 kg) and allotted to 1 of 6 dietary treatments in a randomized complete block design. There were 4 or 5 pigs per pen and 12 pens per treatment.

Prior to diet formulation, representative samples of corn, soybean meal (SBM), and dried distillers grains with solubles (DDGS) were submitted for analysis of CP (AOAC 990.03, 2002), crude fat (AOAC 2003.05, 2006), acid detergent fiber (ADF; ANKOM Technology, 1998), ash (AOAC 942.05, 2012), and neutral detergent fiber (NDF; ANKOM Technology, 2017; Midwest Laboratories, Omaha, NE) in duplicate and for total AA profile analysis by near-infrared spectroscopy (NIR; Ajinomoto Health and Nutrition, Inc., Eddyville, IA) in duplicate (Table 1). Analyzed values were used in diet formulation. Wheat middling samples were not available for analysis prior to initiation of the study, so NRC (2012) loading values were used. Nutrient loading values and standardized ileal digestibility coefficients from NRC (2012) were used for all other ingredients where analytical values were not available.

Treatment diets were formulated in two dietary phases and fed from d 0 to 22 and d 22 to 35, respectively (Table 2 and 3). The six treatments included 2 corn-soybean meal-based diets (Corn-SBM; CS) with either 0 (CS + 0) or 0.01% (CS + 0.01) compound enzyme and 4 corn-

soybean meal-wheat middling-low oil DDGS based diets (Corn-SBM-By-product; CSBP) with 0 (CSBP + 0), 0.01 (CSBP + 0.01), 0.02 (CSBP + 0.02), or 0.03% (CSBP + 0.03) compound enzyme. The CSBP diets were formulated to achieve a targeted 77 kcal/kg reduction in NE and a 1% reduction in CP compared to the CS diets. The 2 CS diets (CS + 0 and CS + 0.01) were formulated to 22.4% CP and 2,399 kcal/kg NE in phase 1 and 20.5% CP and 2,421 kcal/kg NE in phase 2. The 4 CSBP diets (CSBP + 0, CSBP + 0.01, CSBP + 0.02, and CSBP + 0.03) were formulated to 21.3% CP and 2,321 kcal/kg NE in phase 1 and 19.5% CP and 2,344 kcal/kg NE in phase 2. Phase 2 diets included 0.5% titanium dioxide as an indigestible marker. During bagging, complete diet samples were collected from every fourth bag, pooled, ground to reduce particle size, and stored at -20°C.

Throughout the experiment, pig and feeder weights were collected every 7 d to determine ADG, ADFI, and G:F. Feed delivered was recorded for each pen. Fecal samples were collected from 6 pigs (3 barrows and 3 gilts) per treatment d 35 of the study to determine apparent total tract digestibility (ATTD) of dry matter (DM), CP, ADF, and NDF. The pigs selected for collection were the closest to the mean BW of each pen from the middle 6 weight blocks. After collection, fecal samples were dried at 55°C in a forced air oven for 48 h, and fecal DM was determined as the ratio of dried to wet fecal weight.

On d 36, following the completion of the growth study, the same 6 pigs per treatment (36 pigs total) that fecal samples were collected from were euthanized via captive bolt for the collection of ileal digesta and duodenal, jejunal, and ileal tissue samples. Ileal digesta samples were stored at -80°C until freeze dried. Tissue samples were collected and fixed in formalin and submitted to the Kansas State University Veterinary Diagnostic Laboratory for analysis of villus height, crypt depth, and villus height to crypt depth ratio (VH:CD) by a board-certified anatomic

pathologist. A total of 10 measurements were taken on each section of the small intestine from each pig.

Ground feed, fecal, and ileal digesta samples were dried in a 135°C drying oven for 2 h to determine percentage DM of the samples used for titanium analysis (AOAC 985.01, 2007). Titanium dioxide concentration in dried feed, fecal, and ileal digesta samples were determined utilizing procedures outlined by Leone (1973). Fecal, ileal digesta, and phase 2 feed samples were analyzed for ADF (feed and fecal; AOAC 973.18, 2006), NDF (feed and fecal; ANKOM Technology, 2017), and complete AA profile (feed and ileal digesta; AOAC 982.30; AOAC 988.15, 2006) at the University of Missouri Agricultural Experiment Station Chemical Laboratory. Feed and fecal samples were analyzed for CP (AOAC 990.03, 2002) in the Kansas State University Swine Laboratory. The ATTD of DM, CP, ADF, and NDF, and AID of AAs were determined using the index method described by Adeola (2001).

Statistical Analysis

Growth and digestibility 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. Gut morphology data were analyzed as a randomized complete block design with pen as the experimental unit, treatment as a fixed effect, and weight block and pen as random effects to account for 10 observations for each section of small intestine per pig. Linear and quadratic contrasts were constructed with increasing levels of the compound enzyme in the CSBP diets. A pairwise comparison was conducted between the treatments with or without added enzyme within the CS diets. A contrast was constructed to determine the effect of formulation strategy by comparing the CS and CSBP diets with the same enzyme inclusion rates (CS + 0 and CS + 0.01 vs. CSBP + 0 and CSBP + 0.01). Data were analyzed using the lmer package of R (version 4.2.2

(2022-10-31)). Results were considered significant with $P \leq 0.05$ and were considered marginally significant with $P \leq 0.10$.

Results

From d 0 to 22 (phase 1), pigs fed CS diets had increased ($P < 0.05$) ADG and G:F compared to pigs fed CSBP diets, but there were no treatment effects ($P > 0.05$) on ADFI. From d 22 to 35 (phase 2), pigs fed CS diets tended to have increased ($P < 0.10$) G:F compared to pigs fed CSBP diets, but no treatment effects were observed ($P > 0.05$) for ADG or ADFI. There was also a linear decrease ($P < 0.05$) in G:F as enzyme increased in the CSBP diets. From d 0 to 35 (overall), pigs fed CS diets had increased ($P < 0.05$) ADG and G:F compared to pigs fed CSBP diets, but there were no treatment effects ($P > 0.05$) on ADFI.

For intestinal morphology (Table 5), pigs fed CSBP diets had increased ($P < 0.05$) duodenal villus height compared to pigs fed CS diets, but there was no difference ($P > 0.05$) in duodenal crypt depth or VH:CD. There was also a linear decrease ($P < 0.05$) in duodenal villus height and VH:CD as enzyme increased in the CSBP diets, but enzyme addition had no effect ($P > 0.05$) on duodenal crypt depth in the CSBP diets. There was a decrease ($P < 0.05$) in duodenal villus height with added enzyme in the CS diets, but enzyme addition had no effect ($P > 0.05$) on duodenal crypt depth or VH:CD in the CS diets. There were no treatment effects observed ($P > 0.05$) for jejunal or ileal morphology measures.

For ATTD measures (Table 6), pigs fed CS diets had increased ($P < 0.05$) ATTD of DM, CP, and ADF and tended to have increased ($P < 0.10$) ATTD of NDF compared to pigs fed CSBP diets. Enzyme addition had no effect ($P > 0.05$) on ATTD of DM, CP, ADF, or NDF in the CS diets. There was a tendency for a quadratic reduction ($P < 0.10$) in ATTD of DM and CP as enzyme increased in the CSBP diets, but no effect ($P > 0.05$) on ATTD of ADF and NDF.

For AID measures, pigs fed CS diets had increased ($P < 0.05$) AID of Arg, Asp, and Trp and tended to have increased ($P < 0.10$) AID of Gly, His, and Ser compared to pigs fed CSBP diets. There was a tendency ($P < 0.10$) for a reduction in AID of Glu when enzyme was included in the CS diets, but enzyme inclusion in the CS diets had no effect ($P > 0.05$) on AID of the other AAs. There was a quadratic reduction ($P < 0.05$) in AID of Met in the CSBP diets where AID decreased from 0 to 0.02% enzyme but increased from 0.02% to 0.03% enzyme, but enzyme inclusion had no effect ($P > 0.05$) on AID of the other AAs in the CSBP diets. There were no treatment effects on AID of Ala, Cys, Glu, Ile, Leu, Lys, Phe, Pro, Thr, Tyr, or Val.

Discussion

The cell walls of plant derived ingredients can consist of up to 90% non-starch polysaccharides (NSPs; Selvendran and Robertson, 2005). These NSPs can include cellulose, hemicellulose, pectins, α-galactosides, β-glucans, mannans, and xylans (Choct, 1997). Alternative feed ingredients, such as coproducts from cereal grains and oilseeds, when used to reduce feed costs, could result in an increase in NSPs and other ANFs in the diet compared to diets without coproducts (Park and Adeola, 2023). Jaworski et al. (2015) reported total NSP contents (DM basis) of 8.1, 25.0, 9.5, and 30.7% for corn, corn DDGS, wheat, and wheat middlings, respectively. Given the greater content of NSPs in ingredients such as corn DDGS and wheat middlings, NSP-degrading enzymes have the potential to be more effective in low energy, high fiber diets containing cereal grain coproducts (Zijlstra et al., 2010).

Wheat middlings were included at 16% and DDGS were included at 10% of the corn-SBM-by-product diets in the current study to ensure substrate was not limiting and to determine the enzymes' effect on energy and protein utilization. The inclusion of these coproducts achieved the formulation target of approximately 77 kcal/kg reduction in NE and DE and 1% reduction in

CP in the CSBP diets compared to the CS diets. The addition of these coproducts provided substrate, and the reduction in NE and CP allowed us to determine if added enzyme in the CSBP diets would result in similar performance to the CS diets. De Jong et al. (2014) observed a linear reduction in ADG and G:F as NE was reduced by 2.6 and 5.2% in corn-soybean meal-wheat middling based nursery pig diets. The authors observed a 2.7 and 2.9% reduction in ADG and G:F, respectively, as NE was reduced by 2.6%. As NE was reduced by 5.2%, De Jong et al. (2014) observed a 4.8 and 9.7% reduction in ADG and G:F, respectively. In the current study, a 4.0 and 5.2% reduction in ADG and G:F, respectively, was observed as NE was reduced by 3.2%, indicating a similar change in the growth responses relative to energy differences observed by De Jong et al. (2014). Additionally, Tsai et al. (2017) observed decreased ADG, G:F, and ATTD of DM, energy, and CP in pigs fed a high fiber, low energy diet with 30% DDGS compared to pigs fed a corn-soybean meal based diet. This supports the current findings where reduced growth performance was observed with CSBP diets, and a reduction in ATTD of DM, CP, and ADF was observed.

The use of exogenous enzymes is one nutritional strategy to improve growth performance by increasing nutrient utilization and degrading ANFs (Ravindran, 2013). Some exogenous enzymes supplement endogenous enzymes that are present in insufficient amounts, while others are not produced by the pig (Thacker, 2013). Sunzyme, the compound enzyme product in the current study, is comprised of two classes of enzymes, carbohydrases and protease. Carbohydrases are enzymes that break down carbohydrates, including starch and non-starch polysaccharides (Partridge, 2001). Exogenous carbohydrases can include amylase, which supplements endogenous amylase in breaking down starch, and NSP-degrading enzymes, such as xylanase, glucanase, mannanase, galactosidase, cellulase, and pectinase, which are not produced by the pig (Thacker,

2013). These enzymes are substrate specific, meaning there are components in feed ingredients with favorable structure and composition for each enzyme's activity (Park and Adeola, 2023). Xylans and cellulose are the major NSPs in corn, wheat, and their coproducts (Jaworski et al., 2015). Because xylanase is a major component of the compound enzyme, the substrates in the current study complement the enzymatic activity. Tsai et al. (2017) fed xylanase, β-glucanase, and a blend of the two enzymes, which are the two major NSP-enzymes in the current study, in reduced energy and CP diets to nursery pigs. The authors observed increased ADG in pigs fed the enzyme blend compared to pigs fed diets without added enzyme. Additionally, in the early nursery period, the authors observed increased ATTD of DM and CP in pigs fed xylanase or xylanase/β-glucanase blend compared to pigs fed no enzyme and increased ATTD of NDF and ADF in pigs fed xylanase, β-glucanase, or xylanase/β-glucanase blend compared to pigs fed no enzyme. However, in our study, pigs fed xylanase and β-glucanase, in a blend with other enzymes, had similar growth performance and reduced ATTD of DM and CP compared to pigs fed no enzyme. It is important to note that pigs in the study by Tsai et al. (2017) were younger than those used in the current study, indicating there may be more potential for positive effects of enzymes in younger nursery pigs due to inherent enzymatic activity. Additionally, the compound enzyme in the current study included protease along with the carbohydrases. These enzymes could work competitively, and carbohydrases can potentially be degraded when included with protease. Lu et al. (2019) observed decreased ADG and G:F from d 0 to 14 in nursery pigs fed added xylanase compared to the corn-SBM control. However, added xylanase from d 14 to 42 improved performance of pigs fed the corn-SBM control diet from d 0 to 14. The authors hypothesized that the negative effects on performance with added xylanase during the first phase could be due to xylanase reducing the secretion of digestive enzymes by the pig.

Exogenous proteases, in addition to endogenous proteases in pigs, break down proteins and hydrolyze protein based ANFs, which can result in increased nutrient utilization, growth performance, and intestinal health (Song et al., 2022). Park et al. (2020) observed increased ADG, G:F, ATTD of DM and CP, and ileal VH:CD in nursery pigs fed 0.02% of a bacterial derived protease compared to pigs not fed protease in reduced energy and CP diets. However, in the current study, pigs fed bacterial derived protease, in a blend with other enzymes, had similar growth performance and decreased duodenal VH:CD and ATTD of DM and CP compared to those without enzyme supplementation. It is important to note that reduced growth performance and ATTD of DM and CP were observed in pigs fed low energy diets compared to high energy diets by Park et al. (2020), similar to the current study. Also similar to what we observed, Munezero and Kim (2022) found no differences in overall nursery growth performance in pigs fed low CP diets with or without 0.05% of a bacterial protease. However, Munezero and Kim (2022) observed no differences in ATTD of DM and N compared to the decrease observed in the current study with enzyme inclusion. The negative effects on intestinal morphology and nutrient digestibility observed in the current study could potentially be explained by lack of enzyme to digest nutrients that are released as the supplemented enzymes act on ANFs. Additionally, in the current study protease was added with carbohydrases, which could cause competition and degrading of the enzymes themselves.

The previously discussed enzymes can often be added to swine diets in a blend, which could increase their efficacy to degrade a variety of substrates (Park et al., 2016). Similar to the current study, Olukosi et al. (2007) observed no differences in growth performance in pigs fed low energy diets with or without a blend of xylanase, protease, and amylase. However, in contrast with the current study, the authors did observe increased ATTD of DM in pigs fed an

enzyme blend compared to pigs not fed enzymes. It is important to note that Olukosi et al. (2007) fed diets containing 11% wheat middlings and 11% canola meal, providing differences in substrate compared to the current study. Additionally, Kim et al. (2004) observed no differences in growth performance or ATTD of DM and CP in pigs fed diets with or without a blend of protease, pectinase, xylanase, β-glucanase, and amylase; however, substrate could have been limiting considering the low inclusion of 3% wheat and no coproducts in the experimental diets. In contrast to the current study, Koo et al. (2017) observed increased G:F, ileal villus height, duodenal VH:CD, and ATTD of NDF in pigs fed an enzyme blend (cellulase, pectinase, mannanase, galactanase, xylanase, glucanase, amylase, and protease) compared to pigs not fed the enzyme blend in corn-wheat-soybean meal-based diets. These results are in conflict to our findings. However, pigs used in the study by Koo et al. (2007) were newly weaned and had an initial BW of 6.7 kg compared to 13.3 kg in the current study. Therefore, enzymes may be more effective in younger pigs due to differences in their reduced digestive capability. Additionally, Lee et al. (2019) observed increased AID of Ile, Met, and Trp in a linear or quadratic fashion and a tendency for increased AID of Ala, Leu, Phe, Ser, and Val in a linear or quadratic fashion as the level of multi-enzyme (xylanase, β-glucanase, cellulase, amylase, mannanase, pectinase, protease, and invertase) increased in low energy diets. The current study observed reduced AID of Met and no differences in AID of the other AAs when a compound enzyme was added to CSBP diets. However, the inherent AID of AAs in the negative control diet used by Lee et al. (2019) was approximately 10% lower than that of the CSBP in the current study, possibly indicating a greater opportunity for enzymes in diets with lower digestibility of nutrients. The reduction in AID of Met but no differences in the other AAs as enzyme was added to the CSBP diets in the current study can potentially be explained by Met having the highest inherent AID

(93.8%) in the corn-SBM-by-product diet without enzyme. Additionally, after the supplemented enzymes break down the components that are not degradable by the pig, there may be a lack of enzyme to digest the nutrients that are released. Lee et al. (2018) reported increased AID of AAs when mono-component proteases were used but no benefits in AID of AAs when enzyme blends (including proteases and NSP enzymes) were used. This could also explain the lack of differences in AID of most AAs as enzyme was added to the CSBP diets in the current study as the enzymes used could be competing to break down nutrients. Diet composition and inherent digestibility can affect response to enzymes due to differences in substrate and potential for a response to be observed. Enzyme source and composition can also have an effect as enzymes must complement substrate available while not working competitively. The pig's physiological state/endogenous enzymatic capabilities could also contribute to differences in response criteria across studies as enzymes have more potential in younger pigs with less digestive capacity.

In conclusion, results of this study indicate that pigs fed CS diets had increased ADG, G:F, ATTD of DM, CP, and ADF, and AID of Trp and some non-essential AA compared to pigs fed CSBP diets. The inclusion of compound enzymes had no effect on overall growth performance but had a negative impact on duodenal morphology, ATTD of DM and CP, and AID of Met in CSBP diets.

Acknowledgements

Appreciation is expressed to Wuhan Sunhy Biology Co. Ltd. (Wuhan, P.R. China) for partial financial support.

Literature Cited

Adeola, O. 2001. Digestion and balance techniques in pigs. pp. 903. Swine Nutrition, 2nd ed. A. J. Lewis and L. L. Southern ed. CRC Press, Washington, DC.

Adeola, O., and A. J. Cowieson. 2011. BOARD-INVITED REVIEW: Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J. Anim. Sci. 89:3189–3218. doi:10.2527/jas.2010-3715.

ANKOM Technology. 1998. Method for determining acid detergent fiber, ANKOM 200/220 fiber analyzer. Fairport, NY: ANKOM Technology.

ANKOM Technology. 2017. Method for determining neutral detergent fiber, ANKOM 2000 fiber analyzer. Fairport, NY: ANKOM Technology.

AOAC. 2002. Official methods of analysis. 17th ed. Association of Official Analytical Chemists, Arlington, VA.

AOAC. 2006. Official methods of analysis AOAC international. 18th ed. Association of Official Analytical Chemists, Arlington, VA.

AOAC. 2007. Official methods of analysis AOAC international. 18th ed. Association of Official Analytical Chemists, Washington, DC.

AOAC. 2012. Official methods of analysis AOAC international. 19th ed. Association of Official Analytical Chemists, Washington, DC.

Choct, M. 1997. Feed non-starch polysaccharides: Chemical structures and nutritional significance. Feed Mill. Int. 6:13-26.

De Jong, J. A., J. M. DeRouchey, M. D. Tokach, S. S. Dritz, and R. D. Goodband. 2014. Effects of dietary wheat middlings, corn dried distillers grains with solubles, and net energy formulation on nursery pig performance. J. Anim. Sci. 92:3471–3481. doi:10.2527/jas.2013-7350.

Jacela, J. Y., J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. L. Nelssen, D. G. Renter, and S. S. Dritz. 2009. Feed additives for swine: Fact sheets – carcass modifiers, carbohydrate-degrading enzymes and proteases, and anthelmintics. J. Swine Health Prod. 17:325–332.

Jaworski, N. W., H. N. Lærke, K. E. Bach Knudsen, and H. H. Stein. 2015. Carbohydrate composition and in vitro digestibility of dry matter and nonstarch polysaccharides in corn, sorghum, and wheat and coproducts from these grains. J. Anim. Sci. 93:1103. doi:10.2527/jas.2014-8147.

Jha, R., and J. D. Berrocoso. 2015. Review: Dietary fiber utilization and its effects on physiological functions and gut health of swine. Animal. 9:1441–1452. doi:10.1017/S1751731115000919.

Kim, B. G., J. Z. Tian, J. S. Lim, D. Y. Kil, H. Jeon Y., Y. K. Chung, and Y. Y. Kim. 2004. Influences of enzyme complex supplementation on growth, ileal and apparent fecal digestibility and morphology of small intestine in pigs. Asian-Australas. J. Anim. Sci. 17:1729–1735. doi:10.5713/ajas.2004.1729.

Koo, B., J. W. Kim, C. F. M. De Lange, M. M. Hossain, and C. M. Nyachoti. 2017. Effects of diet complexity and multicarbohydrase supplementation on growth performance, nutrient digestibility, blood profile, intestinal morphology, and fecal score in newly weaned pigs. J. Anim. Sci. 95:4060. doi:10.2527/jas2017.1760.

Lee, J. W., R. Patterson, A. Rogiewicz, and T. A. Woyengo. 2019. Nutrient digestibility of multi-enzyme supplemented low-energy and AA diets for grower pigs. J. Anim. Sci. 97:2979–2988. doi:10.1093/jas/skz178.

Lee, S. A., M. R. Bedford, and C. L. Walk. 2018. Meta-analysis: explicit value of mono-component proteases in monogastric diets. Poult. Sci. 97:2078–2085. doi:10.3382/ps/pey042.

Leone, J. L. 1973. Collaborative study of the quantitative determination of titanium dioxide in cheese. AOAC. 56(3):535.

Lu, H., H. Yan, H. M. Masey O'Neill, C. Bradley, M. R. Bedford, P. Wilcock, C. H. Nakatsu, O. Adeola, and K. M. Ajuwon. 2020. Effect of timing of postweaning xylanase supplementation on growth performance, nutrient digestibility, and fecal microbial composition in weanling pigs. Can. J. Anim. Sci. 100:27-36. doi:10.1139/cjas-2019-0021.

Masey O'Neill, H. V., J. A. Smith, and M. R. Bedford. 2014. Multicarbohydrase Enzymes for Non-ruminants. Asian-Australas. J. Anim. Sci. 27:290–301. doi:10.5713/ajas.2013.13261.

Munezero, O., and I. H. Kim. 2022. Effects of protease enzyme supplementation in weanling pigs' diet with different crude protein levels on growth performance and nutrient digestibility. J. Anim. Sci. Technol. 64:854–862. doi:10.5187/jast.2022.e51.

NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington D.C.

Olukosi, O. A., J. S. Sands, and O. Adeola. 2007. Supplementation of carbohydrases or phytase individually or in combination to diets for weanling and growing-finishing pigs1. J. Anim. Sci. 85:1702–1711. doi:10.2527/jas.2006-709.

Park, C. S. and O. Adeola. 2023. Enzymes and Enzyme Supplementation of Swine Diets. In: L. I. Chiba, editor, Sustainable Swine Nutrition. Wiley-Blackwell, Hoboken, NJ. p. 445-469.

Park, C. S., I. Park, and B. G. Kim. 2016. Effects of an enzyme cocktail on digestible and metabolizable energy concentrations in barley, corn, and wheat fed to growing pigs. Livest. Sci. 187:1–5. doi:10.1016/j.livsci.2016.02.003.

Park, S., J. J. Lee, B. M. Yang, J. H. Cho, S. Kim, J. Kang, S. Oh, D.-J. Park, R. Perez-Maldonado, J.-Y. Cho, I.-H. Park, H. B. Kim, and M. Song. 2020. Dietary protease improves growth performance, nutrient digestibility, and intestinal morphology of weaned pigs. J. Anim. Sci. Technol. 62:21–30. doi:10.5187/jast.2020.62.1.21.

Partridge, G. 2001. The Weaner Pig-Enzymes and Biotechnology for the Future. In: M. A. Varley and J. Wiseman, editors, The weaner pig: Nutrition and Management. CAB International, Wallingford, UK. p. 123-152.

R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ravindran, V. 2013. Feed enzymes: The science, practice, and metabolic realities. J. Appl. Poult. Res. 22:628–636. doi:10.3382/japr.2013-00739.

Selvendran, R. R., and J. A. Robertson. 2005. The chemistry of dietary fibre - an holistic view of the cell wall matrix. In: Dietary fibre. Elsevier. p. 27–43. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9781855737785500088.

Song, M., B. Kim, J. H. Cho, H. Kyoung, S. Park, J.-Y. Cho, K. I. Park, H. B. Kim, and J. J. Lee. 2022. Effects of dietary protease supplementation on growth rate, nutrient digestibility, and intestinal morphology of weaned pigs. J. Anim. Sci. Technol. 64:462–470. doi:10.5187/jast.2022.e28.

Thacker, P. A. 2013. Alternatives to antibiotics as growth promoters for use in swine production: a review. J. Anim. Sci. Biotechnol. 4:35. doi:10.1186/2049-1891-4-35.

Tsai, T., C. R. Dove, P. M. Cline, A. Owusu-Asiedu, M. C. Walsh, and M. Azain. 2017. The effect of adding xylanase or β-glucanase to diets with corn distillers dried grains with solubles (CDDGS) on growth performance and nutrient digestibility in nursery pigs. Livest. Sci. 197:46–52. doi:10.1016/j.livsci.2017.01.008.

Woyengo, T. A. 2023. Antinutritional Factors in Feedstuffs. In: L. I. Chiba, editor, Sustainable Swine Nutrition. Wiley-Blackwell, Hoboken, NJ. p. 411-427.

Zijlstra, R. T., A. Owusu-Asiedu, and P. H. Simmins. 2010. Future of NSP-degrading enzymes to improve nutrient utilization of co-products and gut health in pigs. Livest. Sci. 134:255–257. doi:10.1016/j.livsci.2010.07.017.

Table 3.1. Analyzed ingredient composition (as-fed basis)[1]

Nutrient, %	Corn	Soybean meal	DDGS	Wheat middlings
Crude protein	7.86	47.75	28.35	17.30
Crude fat	3.30	1.01	4.18	3.93
Acid detergent fiber	1.55	---	8.75	11.40
Ash	1.04	6.11	5.08	4.89
Neutral detergent fiber	6.70	5.80	24.50	36.10
Essential AAs				
Arginine	0.35	3.37	1.18	---
Histidine	0.20	1.20	0.70	---
Isoleucine	0.25	2.03	1.06	---
Leucine	0.83	3.51	3.06	---
Lysine	0.24	2.88	0.83	---
Methionine	0.18	0.66	0.57	---
Phenylalanine	0.36	2.48	1.40	---
Threonine	0.27	1.88	1.04	---
Tryptophan	0.05	0.62	0.25	---
Valine	0.35	2.15	1.36	---
Non-essential AAs				
Alanine	0.52	2.04	1.92	---
Aspartic acid	0.51	5.42	1.79	---
Cysteine	0.16	0.62	0.52	---
Glutamic acid	1.32	8.74	4.64	---
Glycine	0.31	1.98	1.10	---
Methionine + cysteine	0.33	1.27	1.09	---
Proline	0.47	1.45	2.58	---
Serine	0.36	2.42	1.34	---
Tyrosine	0.14	1.50	1.01	---

[1]Ingredient samples were pooled and submitted for complete proximate analysis in duplicate (Midwest Laboratories, Omaha, NE) and for complete amino acid profile analysis in duplicate (Ajinomoto Health and Nutrition, Inc., Eddyville, IA). Analyzed values for corn, SBM, and DDGS were used in diet formulation. Values from NRC (2012) were used for wheat middlings.

Table 3.2. Ingredient composition of experimental diets (as-fed basis)[1]

	Phase 1		Phase 2	
Ingredient, %	CS	CSBP	CS	CSBP
Corn	60.81	46.59	65.40	51.02
Soybean meal	35.73	23.58	30.97	18.99
Wheat middlings	---	16.00	---	16.00
DDGS	---	10.00	---	10.00
Calcium carbonate	0.92	1.28	0.85	1.21
Monocalcium phosphate	0.79	0.28	0.60	0.10
Sodium chloride	0.60	0.55	0.55	0.50
L-Lys-HCl	0.33	0.59	0.33	0.59
DL-Met	0.18	0.19	0.16	0.17
L-Thr	0.13	0.22	0.12	0.21
L-Trp	0.04	0.07	0.04	0.07
L-Val	0.09	0.16	0.08	0.15
L-Ile	---	0.10	---	0.10
Trace mineral premix	0.15	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25	0.25
Phytase[2]	0.01	0.01	0.01	0.01
Compound enzyme[3]	+/-	+/-	+/-	+/-
Titanium dioxide[4]	---	---	0.50	0.50
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Standardized ileal digestible (SID) amino acids, %				
Lys	1.28	1.28	1.17	1.17
Ile:Lys	60	60	59	59
Leu:Lys	121	114	122	115
Met:Lys	37	37	37	37
Met and Cys:Lys	58	58	58	58
Thr:Lys	64	64	64	64
Trp:Lys	20	20	20	20
Val:Lys	72	72	72	72
His:Lys	38	35	38	35
Total Lys, %	1.43	1.44	1.31	1.32
NE, kcal/kg	2,399	2,321	2,421	2,344
DE, kcal/kg	3,430	3,353	3,415	3,338
SID Lys:NE, g/Mcal	5.34	5.51	4.83	4.99
CP, %	22.4	21.3	20.5	19.5
Ca, %	0.70	0.72	0.62	0.65
STTD P, %	0.43	0.43	0.39	0.39
Ca:P	1.20	1.20	1.20	1.20
ADF, %	3.63	4.42	3.52	4.30
NDF, %	6.1	12.5	6.2	12.6

[1]Phase 1 diets were fed from d 0 to 22, and phase 2 diets were fed from d 22 to 35 of the study.

[2]Sunphase HT (Wuhan Sunhy Biology Co., Ltd., Wuhan, P. R. China) included at 1,227 FTU/kg provided an estimated release of 0.11% STTD P.

[3]Sunzyme (Wuhan Sunhy Biology Co., Ltd., Wuhan, P. R. China) was included at 0 or 0.01% in the corn-SBM diets (CS) and 0, 0.01, 0.02, or 0.03% in the corn-SBM-by-product diets (CSBP).

[4]Titanium dioxide was included at 0.50% of the phase 2 diets as an indigestible marker.

Table 3.3. Analyzed composition of phase 2 experimental diets (as-fed basis)[1]

Formulation strategy:	Corn-SBM		Corn-SBM-By-product			
Nutrient, % Enzyme, %[2]:	0	0.01	0	0.01	0.02	0.03
DM	88.3	88.3	88.5	88.6	88.7	88.8
CP	19.8	20.3	19.5	19.2	19.7	19.5
Acid detergent fiber	3.9	4.0	5.5	6.4	6.9	6.8
Neutral detergent fiber	6.9	8.3	12.7	13.4	14.4	13.9
Essential AAs						
Arginine	1.33	1.30	1.13	1.14	1.07	1.16
Histidine	0.54	0.52	0.50	0.50	0.48	0.50
Isoleucine	0.92	0.90	0.87	0.87	0.84	0.88
Leucine	1.74	1.67	1.64	1.64	1.58	1.63
Lysine	1.33	1.36	1.37	1.33	1.27	1.35
Methionine	0.38	0.45	0.51	0.45	0.38	0.54
Phenylalanine	1.00	0.97	0.88	0.89	0.85	0.89
Threonine	0.86	0.81	0.90	0.80	0.85	0.82
Tryptophan	0.26	0.23	0.23	0.23	0.23	0.26
Valine	1.09	1.08	1.11	1.11	1.06	1.10
Non-essential AAs						
Alanine	1.04	1.00	1.02	1.02	0.99	1.01
Aspartic acid	2.04	1.98	1.65	1.65	1.55	1.67
Cysteine	0.33	0.35	0.35	0.33	0.31	0.37
Glutamic acid	3.81	3.68	3.46	3.47	3.35	3.44
Glycine	0.85	0.84	0.81	0.81	0.76	0.81
Proline	1.18	1.16	1.20	1.23	1.22	1.22
Serine	0.82	0.80	0.73	0.75	0.73	0.75
Tyrosine	0.66	0.63	0.58	0.59	0.56	0.60

[1]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 analyzed for ADF, NDF, and complete AA profile (University of Missouri Agricultural Experiment Station Chemical Laboratory). Samples were analyzed for DM and CP in the Kansas State University Swine Laboratory.

[2]Sunzyme (Wuhan Sunhy Biology Co., Ltd., Wuhan, P. R. China)

Table 3.4. Effect of compound enzymes in diets of different nutrient density on nursery pig growth performance[1]

	Treatment[2]							*P* =			
Formulation strategy:	Corn-SBM		Corn-SBM-By-product					CS vs.	Enzyme	Enzyme in CSBP	
Item Enzyme, %[3]:	0	0.01	0	0.01	0.02	0.03	SEM	CSBP[4]	in CS	Linear	Quadratic
BW, kg											
d 0	13.3	13.3	13.3	13.3	13.3	13.3	0.23	0.983	0.882	0.953	0.811
d 22	28.8	28.5	28.0	27.8	27.6	27.9	0.39	0.005	0.426	0.728	0.324
d 35	40.3	39.9	39.2	39.0	38.4	38.9	0.41	0.002	0.448	0.345	0.251
d 0 to 22 (Phase 1)											
ADG, g	702	687	662	659	649	665	11.6	0.004	0.339	0.978	0.395
ADFI, g	1,042	1,026	1,049	1,035	1,014	1,049	18.6	0.641	0.487	0.800	0.129
G:F, g/kg	674	670	632	638	641	634	6.4	< 0.001	0.616	0.741	0.281
d 22 to 35 (Phase 2)											
ADG, g	883	866	863	847	836	849	14.4	0.175	0.394	0.397	0.304
ADFI, g	1,634	1,570	1,617	1,625	1,591	1,702	33.9	0.570	0.186	0.153	0.134
G:F, g/kg	542	553	535	521	530	500	10.8	0.087	0.455	0.047	0.457
d 0 to 35 (Overall)											
ADG, g	769	750	736	729	718	733	10.2	0.010	0.206	0.683	0.279
ADFI, g	1,261	1,222	1,258	1,254	1,228	1,292	21.5	0.489	0.199	0.422	0.111
G:F, g/kg	610	615	586	582	587	568	7.2	< 0.001	0.665	0.152	0.291

[1]A total of 355 nursery pigs (DNA 241 × 600, initially 13.3 ± 0.23 kg BW) were used in a 35-d growth trial with 4-5 pigs per pen and 12 replications per treatment.

[2]Corn-SBM diets (CS) were corn-soybean meal based and formulated to 22.4% CP and 2,399 kcal/kg NE in phase 1 and 20.5% CP and 2,421 kcal/kg NE in phase 2. Corn-SBM-by-product diets (CSBP) were corn-soybean meal-wheat middling-low oil DDGS based and formulated to 21.3% CP and 2,321 kcal/kg NE in phase 1 and 19.5% CP and 2,344 kcal/kg NE in phase 2.

[3]Sunzyme, Wuhan Sunhy Biology Co., Ltd., Wuhan, P. R. China.

[4]Corn-SBM vs. Corn-SBM-by-product diets with the same enzyme inclusion rates (CS + 0 and CS + 0.01 vs. CSBP + 0 and CSBP + 0.01)

Table 3.5. Effect of compound enzymes in diets of different nutrient density on nursery pig intestinal morphology[1]

	Treatment[2]							*P* =			
Formulation strategy:	Corn-SBM		Corn-SBM-By-product					CS vs.	Enzyme	Enzyme in CSBP	
Item Enzyme, %[3]:	0	0.01	0	0.01	0.02	0.03	SEM	CSBP[4]	in CS	Linear	Quadratic
Duodenum											
Villus height, μm	622	528	715	604	633	567	33.9	0.003	0.017	0.001	0.403
Crypt depth, μm	566	517	548	536	540	570	29.3	0.998	0.231	0.576	0.466
VH:CD[5]	1.16	1.13	1.33	1.17	1.22	1.03	0.106	0.197	0.813	0.028	0.888
Jejunum											
Villus height, μm	667	636	694	682	665	652	52.1	0.490	0.675	0.547	0.987
Crypt depth, μm	369	380	402	353	390	393	24.4	0.892	0.725	0.920	0.229
VH:CD	1.88	1.75	1.79	2.14	1.76	1.74	0.196	0.400	0.588	0.494	0.302
Ileum											
Villus height, μm	473	429	453	379	428	415	23.7	0.151	0.192	0.538	0.212
Crypt depth, μm	236	228	224	238	239	234	16.7	0.962	0.730	0.675	0.599
VH:CD	2.29	1.98	2.09	1.72	1.91	1.85	0.167	0.171	0.188	0.478	0.363

[1]A total of 355 nursery pigs (DNA 241 × 600, initially 13.3 ± 0.23 kg BW) were used in a 35-d growth trial with 4-5 pigs per pen and 12 replications per treatment. Tissue samples were collected from the duodenum, jejunum, and ileum from 6 pigs per treatment.

[2]Corn-SBM diets (CS) were corn-soybean meal based and formulated to 22.4% CP and 2,399 kcal/kg NE in phase 1 and 20.5% CP and 2,421 kcal/kg NE in phase 2. Corn-SBM-by-product diets (CSBP) were corn-soybean meal-wheat middling-low oil DDGS based and formulated to 21.3% CP and 2,321 kcal/kg NE in phase 1 and 19.5% CP and 2,344 kcal/kg NE in phase 2.

[3]Sunzyme, Wuhan Sunhy Biology Co., Ltd., Wuhan, P. R. China.

[4]Corn-SBM vs. Corn-SBM-by-product diets with the same enzyme inclusion rates (CS + 0 and CS + 0.01 vs. CSBP + 0 and CSBP + 0.01)

[5]VH:CD = villus height to crypt depth ratio

Table 3.6. Effect of compound enzymes in diets of different nutrient density on nursery pig nutrient digestibility[1]

	Treatment[2]							*P* =			
Formulation strategy:	Corn-SBM		Corn-SBM-By-product					CS vs.	Enzyme	Enzyme in CSBP	
Item Enzyme, %[3]:	0	0.01	0	0.01	0.02	0.03	SEM	CSBP[4]	in CS	Linear	Quadratic
ATTD, %											
DM	87.6	86.5	81.8	78.7	79.0	79.6	0.97	< 0.001	0.426	0.132	0.054
CP	84.7	84.3	82.6	80.4	80.4	82.5	1.22	0.012	0.763	0.959	0.065
ADF	52.0	50.1	37.0	38.2	41.0	38.2	5.48	0.020	0.809	0.792	0.717
NDF	43.3	46.2	39.3	28.8	36.1	30.7	5.50	0.060	0.709	0.452	0.643
AID, %											
Essential AAs											
Arg	93.9	92.8	91.0	90.8	89.6	90.5	0.95	0.012	0.394	0.508	0.581
His	90.9	89.0	87.8	87.2	85.8	86.4	1.43	0.073	0.307	0.360	0.657
Ile	89.3	87.9	87.5	87.5	85.6	86.6	1.32	0.380	0.428	0.396	0.714
Leu	88.7	87.5	87.2	87.5	84.7	86.4	1.55	0.633	0.566	0.427	0.621
Lys	91.2	90.0	89.9	89.5	88.2	89.2	1.20	0.400	0.458	0.520	0.510
Met	91.7	92.9	93.4	92.8	89.3	93.1	0.93	0.324	0.303	0.264	0.015
Phe	89.4	87.7	86.8	86.7	84.4	85.8	1.50	0.202	0.388	0.413	0.593
Thr	86.6	83.7	85.0	82.6	82.2	81.7	1.87	0.467	0.252	0.195	0.572
Trp	92.1	90.2	88.1	87.9	88.0	88.9	1.58	0.050	0.407	0.695	0.726
Val	88.2	86.7	86.4	86.2	84.5	85.1	1.51	0.434	0.465	0.383	0.750
Non-essential AAs											
Ala	86.3	84.0	84.6	85.1	81.6	83.0	1.89	0.869	0.377	0.305	0.804
Asp	89.6	86.4	84.9	85.1	82.5	83.9	1.51	0.048	0.132	0.407	0.689
Cys	83.1	79.9	79.5	78.1	73.7	78.5	2.43	0.265	0.343	0.499	0.204
Glu	92.1	89.2	89.5	90.2	87.6	89.0	1.21	0.507	0.082	0.433	0.739
Gly	85.5	81.2	79.4	78.1	75.4	76.8	3.20	0.082	0.248	0.371	0.596
Pro	88.4	86.7	86.4	87.3	84.5	85.3	1.68	0.662	0.445	0.396	0.982
Ser	88.6	87.2	84.9	84.9	83.5	84.1	1.63	0.058	0.499	0.572	0.818
Tyr	90.4	88.2	87.3	87.3	85.4	86.8	1.41	0.153	0.245	0.561	0.617

[1]A total of 355 nursery pigs (DNA 241 × 600, initially 13.3 ± 0.23 kg BW) were used in a 35-d growth trial with 4-5 pigs per pen and 12 replications per treatment. Fecal and ileal digesta samples were collected from 6 pigs per treatment.

[2]Corn-SBM diets (CS) were corn-soybean meal based and formulated to 22.4% CP and 2,399 kcal/kg NE in phase 1 and 20.5% CP and 2,421 kcal/kg NE in phase 2. Corn-SBM-by-product diets (CSBP) were corn-soybean meal-wheat middling-low oil DDGS based and formulated to 21.3% CP and 2,321 kcal/kg NE in phase 1 and 19.5% CP and 2,344 kcal/kg NE in phase 2.

[3]Sunzyme, Wuhan Sunhy Biology Co., Ltd., Wuhan, P. R. China.

[4]Corn-SBM vs. Corn-SBM-by-product diets with the same enzyme inclusion rates (CS + 0 and CS + 0.01 vs. CSBP + 0 and CSBP + 0.01).