Effects of increasing AA ratios, SBM and DDGS on performance and carcass characteristics of finishing pigs and the inclusion of HiPhorius Phytase on nursery and finishing pig growth performance, carcass characteristics, serum chemistry, and bone mineralization

by

Macie Elizabeth Reeb

B.S., Kansas State University, 2021

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Science
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2023

Approved by:

Co-Major Professor
Robert Goodband

Approved by:

Co-Major Professor
Jason Woodworth

Copyright

© Macie Reeb 2023.

Abstract

This thesis includes three chapters addressing very practical questions facing swine nutritionists including replacing soybean meal with dried distillers grain with solubles and feed-grade amino acids and the addition of a novel phytase source in nursery and finishing pigs. Two experiments using a total of 1,701 finishing pigs was used to determine the effect of amino acid adjustments, soybean meal and dried distillers grain with solubles inclusion on performance and carcass characteristics of finishing pigs. In Exp.1 no effect was observed on growth performance based on dietary amino acid adjustment. In Exp. 2, an improvement in feed efficiency was observed in pigs fed dried distillers grain with solubles -based diets with an amino acid adjustment. Additionally, two experiments using a total of 2,321 pigs were conducted to evaluate the inclusion of HiPhorius phytase on growth performance, serum chemistry, carcass characteristics, and bone mineralization o finishing pigs. Decreased growth performance (Exp.1) as well as bone mineralization (Exp.2) was observed, indicating that the phytase release provided by the phytase was less than the assumed release used in diet formulation. Finally, another experiment with 297 nursery pigs was conducted to determine the effects of HiPhorius phytase on nursery pig growth performance and bone mineralization. Increasing phytase in diets formulated below the pig's P requirement improved growth performance and bone mineralization. Increasing phytase in diets with 0.27% aP did not influence growth performance, but improved bone mineralization. In summary, these experiments provide data on the efficacy of a new phytase source in nursery and finishing pigs, and additional insight on the interrelationship of branch-chain amino acids and large neutral amino acids in diets containing dried distillers grain with solubles in finishing pigs.

Table of Contents

List of Tables

Acknowledgements

I would like to thank my committee members for their continued support and guidance. It has truly been an honor and a privilege to get to learn from some of the best swine nutritionists in the industry. Each professor shared their expertise with me and aided in my growth and learning. K-State considers our graduate office the applied swine nutrition *team*, and it truly is a team.

I would also like to thank my fellow graduate students for all of their help in and out of the office, at the farm, or at the feed mill. I cannot wait to see how big of an impact the people from our office make in the swine industry in the coming years.

To my family and friends: THANK YOU will never be enough to let you know how grateful I am for your love and support. I would not have been able to make it through these last two years without the amazing friendships I have formed in my time in Manhattan over the last 6 years. Mom, Dad, and Rachel, I am blessed to get to call you my parents. I am a product of the lessons you have taught me and continue to teach me. Thank you for instilling in me a desire to learn and grow and to work hard in everything I do. The
the sacrifices that you have all made to ensure that I had
the most amazing opportunities in life did not go unnoticed. Sav – we are the kind of sisters who don't really hug, and don't talk every day, but couldn't live without each other. Thank you for understanding my goofy humor and encouraging me to learn from your mistakes and sometimes to make new ones of my own.

Last and most importantly, I would like to thank God for giving me the strength to face the challenges life throws at all of us and sticking by my side through it all. Isaiah 41:10.

Chapter 1 - Determining the effect of amino acid adjustments, soybean meal and dried distillers grain with soluables inclusion on growth performance and carcass character

Abstract

Two experiments were conducted to determine the interrelationship of branch-chain amino acids (BCAA) and large neutral amino acids in diets containing dried distillers grain with solubles (DDGS) in finishing pigs. In Exp.1, 621 pigs (DNA 241 × 600; initially 62.6 ± 0.46 kg) were used in a 65-d study. The 6 dietary treatments were arranged in a 3 × 2 factorial with main effects of SBM level (low, medium, high) and Val:Lys and Trp:Lys ratios (standard or high). There were 8 or 9 pigs per pen and 12 replications per treatment. There was no evidence ($P >$ 0.05) of SBM × AA ratio interactions or treatment differences for any response criteria from 63 to 109 kg. From 109 to 136 kg, a marginally significant ($P = 0.084$) SBM × AA ratio interaction was observed for average daily gain (ADG). The medium level of SBM with standard Val:Lys and Trp:Lys ratios resulted in greater (quadratic, $P < 0.01$) ADG and gain to feed (G:F) compared to other SBM levels. There were no significant differences observed in overall ADG or average daily feed intake (ADFI). A marginally significant SBM × AA ratio interaction ($P =$ 0.052) was observed for overall G:F. Increasing SBM in diets with high Val:Lys and Trp:Lys ratios decreased G:F ($P = 0.049$), while there was no difference observed for pigs fed diets with standard Val:Lys and Trp:Lys ratios. In Exp. 2, 1,080 pigs (PIC 337 × 1050, initially 26.5 ± 0.57 kg) were used in a 121-d experiment to determine the effects of added SBM versus using an AA adjustment in diets with dried distillers grains with solubles (DDGS) on growth performance and carcass characteristics. There were 27 pigs per pen and 10 replications per treatment**.** Treatments

diets consisted of: (1) A control diet containing high SBM with no DDGS, (2) DDGS-based diet with a medium level of SBM, (3) DDGS-based diet with low SBM + Val, Ile, and Trp to equal levels as in diet 2, and 4) Treatment 3 but without the Val, Ile, and Trp adjustment (still meeting requirement estimates for all AA). Pigs fed DDGS-based diets had decreased ADG ($P = 0.014$) and hot carcass weight ($P = 0.018$) than pigs fed the control diet. There was an increase in G:F ($P < 0.05$) for pigs fed the high SBM diet without DDGS as compared to pigs fed diets including DDGS and low levels of SBM with no AA adjustment. In conclusion, the response to dietary BCAA level was variable. The results of these studies were inconsistent in the response to AA adjustments in DDGS-containing diets with high Leu:Lys. Additional research evaluating these AA level interactions is needed to predict the growth responses more accurately.

Key Words: branched-chain amino acids, dried distillers grains, finishing pigs, growth, soybean meal

List of abbreviations

ADG, average daily gain

ADFI, average daily feed intake

BCAA, branched-chain amino acid

BW, body weight

DDGS, dried distillers grain solubles

G:F, gain-to-feed

HCW, hot carcass weight

LNAA, large neutral amino acids

NE, net energy

SBM, soybean meal

SID, standardized ileal digestibility

STTD, standard total tract digestibility

Introduction

Branch-chain amino acids (BCAA) and large neutral amino acids (LNAA) have a complicated relationship in swine diets. Branch chain amino acids are a collective group of structurally similar amino acids (AA) comprised of Ile, Leu, and Val, all of which also share the same first steps in catabolism (Harris et al., 2005). Leucine is the most potent stimulator of branch-chain amino acid transferase (BCAT), which is responsible for the first step of BCAA catabolism (Harper et al., 1984). Excess of any one BCAA leads to an increase in catabolism of all the BCAA. Wessels et al. (2016) observed that feeding diets high in Leu increased Leu and reduced Ile and Val concentrations in plasma and several other tissues by increased activity of the branched-chain alpha-ketoacid dehydrogenase complex (BCKD).

Branch-chain amino acids and LNAA also share common transporters into the brain (Pardridge et al. 1977). Research has shown that high concentrations of Leu can decrease the absorption of LNAA, such as Trp, which is a precursor of serotonin and can impact feed intake (Kwon et al. 2022). However, increasing Trp in high Leu diets has been shown to help overcome the negative performance from the high dietary Leu (Kwon et al. 2022). Cemin et al. (2009) developed a growth prediction model suggesting that the addition of different combinations of Ile, Val, and Trp can help recover decreased growth performance in diets with high Leu:Lys ratios.

It is often economical to include DDGS in the diet, which also allows higher levels of feed-grade amino acids (AA) to be used and reduces the level of soybean meal in the diet. In diets containing 30% DDGS for growing pigs, dietary standard ileal digestible (SID) Leu will exceed the requirement by 50 to 100% (Kwon et al., 2022). Because DDGS will increase Leu, it might be beneficial to increase BCAA or LNAA to maintain performance (Kerkaert et al., 2020).

Therefore, our objective was to determine the interrelationship of branch-chain amino acids and large neutral amino acids in finishing pig diets containing DDGS. Our hypothesis was that adding increased levels of BCAA and LNAA to diets containing high Leu from DDGS would improve pig performance.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in these experiments (4485 and 4375). Experiment 1 was conducted at the Kansas State University Swine Teaching and Research Center, Manhattan, KS. The facility is mechanically ventilated, and each pen is equipped with a 2-hole stainless steel dry self-feeder and a bowl waterer for *ad libitum* access to feed and water. Pigs were provided 0.73 m^2 of floor space per pig. Experiment 2 was conducted at a commercial research-finishing site in southwest Minnesota. The barn was mechanically ventilated and double-curtain-sided. Each pen was equipped with a 5-hole stainless steel dry self-feeder and a bowl waterer for *ad libitum* access to feed and water. Pigs were provided 0.62 m^2 of floor space per pig until 120 kg and then 0.73 m^2 until the remaining pigs were marketed. In both studies, daily feed additions to each pen were accomplished using a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) able to record the amount of feed provided for individual pens. Pigs were weighed approximately every 14 days to determine ADG, average daily feed intake (ADFI), and gain-to-feed ratio (G:F).

Experiment 1

Two groups of approximately 310 pigs (Group 1: 303 pigs, Group 2: 318; 621 pigs total; DNA 241 × 600; initially 62.6 ± 0.46 kg) were used in a 61-d growth trial to determine the effect of increasing soybean meal (SBM) and Val:Lys and Trp:Lys ratios in diets containing 25%

DDGS on finishing pig performance. Pens of pigs were assigned to 1 of 6 dietary treatments in a randomized complete block design with BW as a blocking factor. Treatments were arranged in a 3 × 2 factorial with main effects of SBM level (low, medium, high) and Val:Lys and Trp:Lys ratios (standard or high). Dietary treatments were fed in meal form in three BW phases from approximately 63 to 82, 82 to 109, and 109 to 136 kg. There were 8 or 9 pigs per pen and a total of 12 pens per treatment. On the last day of the trial, final pen weights were obtained, and all pigs were transported to a U.S. Department of Agriculture-inspected packing plant (Triumph Foods, St. Joe, MO).

Samples of the corn, DDGS, and SBM were analyzed for DM, CP, and AA profile (Ajinomoto Animal Nutrition North America, Inc., Eddyville, IA). Standard procedures from AOAC (2006) were followed for analysis of moisture (Method 934.01), CP (Method 990.03), and AA profile (method 994.12; AOAC International, 2012). Analyzed amino acid concentrations and SID coefficients (NRC, 2012) were used in diet formulation (Table 1).

All diets were manufactured at the Hubbard Feeds feed mill in Beloit, Kansas and subsequently delivered to the Kansas State University Teaching and Research Center, Manhattan, KS. Diets were formulated to 0.85, 0.74, 0.65% SID Lys (during phases 1, 2, and 3 respectively) with the other AA set to meet or exceed (NRC 2012) requirement estimates (Tables 2, 3, and 4). Complete diet samples were taken from 6 feeders of each treatment at the beginning of each phase change and pooled into one homogenized sample per dietary treatment.

Experiment 2

A total of 1,080 pigs (PIC 337 × 1050, initially 26.5 ± 0.57 kg) were used in a 121-d experiment to determine the effects of added SBM versus using an AA adjustment in diets with

dried distillers grains with solubles (DDGS) on growth performance and carcass characteristics**.** Pens of pigs were randomly assigned to 1 of 4 dietary treatments in a completely randomized design. Treatments consisted of: (1) A control diet containing high SBM with no DDGS, (2) DDGS-based diet with a medium level of SBM, (3) DDGS-based diet with low SBM + (Val, Ile, and Trp to equal levels in diet 2, and 4) Treatment 3 but without the Val, Ile, and Trp adjustment (still meeting requirement estimates for all AA). The AA adjustment more specifically included additional Val, Ile and Trp so that the individual AA ratios for Val:Lys, Ile:Lys, and Trp:Lys within treatment 3 (DDGS + low SBM) matched that of the medium SBM diet (Treatment 2) and the Ile+Val+Trp:Leu ratio was equal to that of the high SBM diet (control diet) (Tables 5, 6, 7, and 8). There were 27 pigs per pen and 10 replications per treatment.

Dietary treatments were fed in 4 phases. Diets were formulated to 1.20, 0.99, 0.83, 74% SID Lys (during phases 1, 2, 3 and 4 respectively). Phase 1 was fed from 23 to 45 kg, phase 2 from 45 to 73 kg, phase 3 from 73 to 100 kg, and phase 4 from 100 to 136 kg. All diets were fed in meal form and manufactured at the New Horizon Farms Feed Mill in Pipestone, MN. All diets were formulated to meet or exceed NRC (2012) requirement estimates for all nutrients. Complete diet samples were taken during each finishing phase. Samples were stored at -20°C until they were submitted for analysis of DM, CP, Ca and P (Midwest Laboratories, Omaha, NE). Standard procedures from AOAC (2006) were followed for analysis of moisture (Method 934.01), CP (Method 990.03), Ca and P (Method 985.01).

On d 99, the 4 heaviest pigs in each pen were selected and marketed. These pigs were included in growth performance data but not in the final pen carcass data. On the last day of the trial, final pen weights were obtained, and the remaining pigs were tattooed with a pen identification number and transported to a U.S. Department of Agriculture-inspected packing

plant (JBS Swift, Worthington, MN) for carcass data collection. Carcass measurements included hot carcass weight (HCW), loin depth, backfat, and percentage lean. Percentage lean was calculated from a plant proprietary equation. Carcass yield was calculated by dividing the pen average HCW by the pen average final live weight obtained at the farm.

Statistical Analysis

For Experiment 1, data were analyzed as a randomized complete block design for a 3 × 2 factorial using the lmer function from the lme4 package in R (version 3.5.2 (02-07-2018), R Foundation for Statistical Computing, Vienna, Austria) with pen serving as the experimental unit and weight block as a random effect. Pre-planned contrast statements were used to evaluate the dose response interaction of SBM level and Val:Lys and Trp:Lys ratios, as well as the main effects of SBM level and Val:Lys and Trp:Lys ratios.

For Experiment 2, data were analyzed as a completely randomized design for one-way ANOVA using the lmer function from the lme4 package in R (version 3.5.2 (02-07-2018) with pen as the experimental unit and treatment considered fixed effect for all performance criteria. Lean percentage, loin depth, and backfat depth considered HCW as a covariate in the model, and data was analyzed at a pen level. Besides comparing individual treatments, a contrast was used to compare the response to the control diet to the mean of the three diets containing DDGS. A Tukey multiple comparison adjustment was used to control Type I error rate. All results were considered significant with P-values ≤ 0.05 and considered marginally significant with P-values > 0.05 and ≤ 0.10.

Results

Experiment 1

For phases 1 and 2 and overall, no SBM × AA ratio interactions were observed ($P > 0.05$; Table 9). In phases 1 and 2, no evidence for treatment differences ($P > 0.10$) were observed for ADG, ADFI, and G:F. However, in phase 3, a marginally significant SBM × AA ratio interaction was observed for ADG ($P = 0.084$). The medium level of SBM with standard Val:Lys and Trp:Lys ratios resulted in greater ADG compared to other SBM levels (quadratic, $P = 0.003$), while no difference in ADG was observed with increasing SBM when Val:Lys and Trp:Lys ratios were increased ($P = 0.501$). Additionally, G:F was improved for pigs fed the medium level of SBM in phase 3 (quadratic, $P = 0.007$). In spite of the improvement observed in phase 3, there were no differences ($P > 0.10$) observed in overall ADG or ADFI, but a marginally significant SBM × AA ratio interaction was observed for overall G:F ($P = 0.052$). Increasing SBM in diets with high Val:Lys and Trp:Lys ratios resulted in decreased G:F ($P = 0.049$), whereas no difference in overall G:F was observed when increasing SBM with standard Val:Lys and Trp:Lys ratios ($P = 0.435$).

Using the meta-regression model and equations provided by Cemin et al. (2019), relative differences between the predicted and observed performance of these pigs were calculated (Table 10). Comparing predicted and actual responses, the model accurately predicted the performance for pigs fed diets with standard AA ratios with all performance criteria being between 98 and 102% of predicted; however the model tended to overestimate performance for increased Val:Lys and Trp:Lys ratios (as indicated by % of predicted ADG and G:F being 96 to 98% of actual).

Experiment 2

During the grower phase, d 0 to 56, pigs fed high SBM diets with no DDGS had increased ADG ($P = 0.001$) and G:F ($P = 0.001$) compared to those fed diets containing DDGS (Table 11). On d 56, pigs fed high levels of SBM without DDGS had increased BW ($P < 0.05$) compared to pigs fed low levels of SBM with no additional AA, with pigs fed the other two treatments intermediate. However, during the finisher phase, d 56 to 121, there were no significant differences observed on any performance criteria ($P > 0.10$).

Overall, pigs fed DDGS-based diets had decreased ADG ($P = 0.014$) compared to pigs fed the corn-SBM control diet. Pigs fed the high SBM diet without DDGS had increased G:F ($P < 0.05$) compared to pigs fed low levels of SBM and no AA adjustment, with pigs fed the other two diets intermediate.

For carcass characteristics, there was a tendency ($P = 0.074$) for a difference in HCW between treatments. Pigs fed the high SBM diet without DDGS had increased HCW ($P = 0.018$) compared to pigs fed diets containing DDGS. There were no statistically significant ($P > 0.10$) differences between treatment in any of the other carcass traits.

Using Cemin's meta-regression model, again comparing predicted and actual responses, the model over predicted the ADG and G:F response to the amino acid adjustment and medium SBM diet containing DDGS with ADG and G:F criteria being 97 to 98% of predicted (Table 12).

Discussion

Soybean meal is an ingredient commonly used in swine diets due to its high CP and balanced AA profile. Dried distillers grain with solubles is also routinely found in US swine diets as an alternative protein and AA source and can typically help lower diet costs by partially replacing SBM. However, it is important to consider that the Leu:Lys ratio increases when

including DDGS as compared to SBM. It is well documented that Trp, Val, and Ile can become limiting when feed-grade amino acids are included at higher levels in corn–SBM diets or corn-SBM-DDGS diets, while Leu is in excess due to its high concentration in corn and corn by-products (NRC, 2012).

Extensive research efforts have focused on understanding the utilization of BCAA and LNAA in swine diets. Studies show that feeding diets high in Leu can negatively impact performance (Kwon et al., 2022). Research has also shown the importance of adjusting or increasing Val, Ile, and Trp in relation to Leu to maintain performance (Kerkaert et al., 2020; Clizer et al., 2021). Excess Leu will increase the catabolism of all BCAA, potentially leading to deficiencies in Ile and Val, as well as Trp. High Leu concentrations also can overstimulate the mTOR signaling pathway, resulting in an inhibitory effect on feed intake (Cota et al., 2006). When BCAA and Trp are impacted due to excess Leu, additional SBM or feed-grade AA of those specific amino acids can be used to help recover growth performance by increasing their levels relative to Leu (Cemin et al., 2019).

Cemin et. al., (2019) developed a growth prediction model that suggested a negative impact on ADG was observed when Leu:Lys was elevated. This was due to a reduction in ADFI and G:F because of insufficient levels of BCAA and LNAA relative to Leu. The model predicted that the addition of Val, Ile, or Trp alone or in combination could counteract the negative effects of high Leu on growth performance. This model was used as guidance to determine the inclusion rates for Trp and Val in high AA diets in Exp. 1. Based on this model, the high Leu content from corn and DDGS in diets with standard AA ratios was thought to increase catabolism of all BCAA, and therefore some AA deficiencies could develop and increased SBM would be needed to increase performance. In diets where Val:Lys and Trp:Lys ratios were adjusted for this higher

Leu content, the additional SBM might not be needed to further improve performance as it is not likely that these two amino acids would become deficient. Using the meta-regression model and equations provided by Cemin et al. (2019), relative differences between the predicted and observed performance of these pigs were calculated. Looking at both experiments, the model tended to over predict performance of pigs with an BCAA adjustment. In Exp.1 the model's overestimation of performance for pigs fed increased Val:Lys and Trp:Lys ratios is a result of pigs having lower intake and gain compared to what was expected using the model. Similarly, in Exp.2, pigs had decreased ADG and G:F relative to the models predictions.

In Exp. 1 it was hypothesized that increasing the SBM in diets formulated using standard AA ratios for Val:Lys and Trp:Lys would bring in additional AA and therefore potentially recover lost growth performance. However, increasing SBM to increase Val:Lys and Trp:Lys did not influence pig growth compared to when those AA's were provided directly from feed-grade amino acids. While no differences in growth were found in Phases 1 and 2, a quadratic improvement in ADG was observed in phase 3, where gain was increased with up to 4% SBM inclusion in both standard and high AA ratio diets. Regardless of AA formulation, an improvement in G:F was seen in the third phase as well with increasing SBM. Overall, there was also an unexpected linear decrease in G:F for pigs fed diets with high AA ratios, however the numerical difference was only a decrease of 1%. This study indicates that 4% SBM in the late finishing period was needed in the presence of 25% DDGS to help maximize performance. This is in agreement with Holen et al. (2023) where three studies were conducted to determine the effects of increasing soybean meal (SBM) levels by replacing feed-grade amino acids (AA) in corn, corn dried distillers grains with solubles (DDGS), and corn-wheat midds-based diets on growth performance of late finishing pigs. The combined results of Holen et al. (2023) suggest

that inclusion of at least 4% to 8% dietary SBM at the expense of feed-grade amino acids in corn-based diets with or without grain coproducts can improve growth performance (specifically G:F) in late-finishing.

Low crude protein (CP), AA fortified diets are a well-established practice in the swine industry to reduce cost and nitrogen excretion. It is important to still retain adequate CP in later finishing (≥13.5%), as it affects pig growth and development (Soto et al., 2019). When the CP level is too low, there is not enough nitrogen to synthesize non-essential AA, and therefore it can negatively influence performance, regardless of increasing feed-grade AA in the diet. The CP of the low SBM diet in phase 3 was 13.7%, which should have been adequate based on Soto et al. (2019). Thus, the responses seen in later finishing do not appear to be attributed to CP levels during that phase. The results of this study showed that contrary to our hypothesis, there was little effect of increasing Val:Lys and Trp:Lys ratios in DDGS-based diets. A potential explanation is that the diets were over-formulated, meaning AA ratios were formulated already at or above NRC (2012) requirements in the standard AA diets. This prevented any further improvements when AA ratios were increased, leading to the lack of significant responses and conflicting with results observed by Cemin et al. (2019) as well as the results of Exp 2. There was no further improvements in intake or gain when AA ratios were increased in diets compared to when pigs were fed standard AA ratios. This could also provide somewhat of an explanation as to why Cemin's model tended to overestimate the performance of pigs fed increased AA ratios.

In Experiment 2, pigs fed the diet with no DDGS had improved growth from d 0 to 56 but not 56 to 121 compared to pigs fed diets containing DDGS. This might be partially explained by the level of DDGS having been decreased from 25 to 15% in the last feeding phase. This data

also showed, unlike in Exp. 1, improvements in growth resulting from increasing AA ratios relative to Leu when including higher DDGS in the diets. This study shows improvements in feed efficiency when pigs are fed high SBM diets compared to the pigs fed diets containing DDGS with low SBM and no additional feed-grade AA provided. This more closely aligns with previous findings that showed performance could be somewhat recovered by increasing other BCAA and Trp in DDGS-based diets with high Leu (Cemin et al., 2019; Clizer et al., 2021; Kerkheart et al., 2021). However, similar to Exp. 1, Cemin's model overpredicted the ADG and G:F response to the amino acid adjustment and medium SBM diet containing DDGS with ADG and G:F criteria being 97 to 98% of predicted. Also similar to Exp.1, in Exp.2 significant responses were not found in late finishing when lower dietary DDGS levels were fed. Comparing the Leu:Lys ratios of diets with DDGS in Exp. 2 to Exp. 1 (all contained 25% DDGS), diets in Exp. 2 also had consistently lower Leu:Lys ratios. Looking specifically at Exp. 2, pigs fed the DDGS diet with medium SBM level had decreased performance compared to expectations, considering the diet had almost the same AA profile to that of the high SBM (control) diet. However, the Leu:Lys ratio was higher in this treatment than the low SBM diets, which could help to explain the decreases in growth performance. Furthermore, looking at intake/d for each study, pigs in Exp. 1 had greater ADFI compared with pigs in Exp. 2 (2.98 kg/d and 2.55 kg/d, respectively). Thus it could be argued that in Exp. 1 because pigs on standard AA diets were consuming a higher volume of feed and thus more energy and amino acids, there was no opportunity for additional Val and Trp to improve performance.

The results of these studies were inconsistent in the response to AA adjustments in high Leu:Lys diets resulting from including DDGS. However, some benefits were observed, specifically improvements in G:F, when increasing BCAA and Trp in high Leu diets, either with

a higher inclusion of SBM or additional feed-grade AA. Additional research evaluating these AA level interactions is needed to determine when increased BCAA and LNAA are required in diets containing DDGS.

References

AOAC. 2023. Official methods of analysis AOAC international. 22nd ed. Association of Official Analytical Chemists, Arlington, VA.

Anderson, B. E. 2021. Impact on performance and carcass characteristics when replacing soybean meal with distillers dried grains with solubles and crystalline amino acids in diets of growing and finishing pigs. M.S Thesis. North Carolina State University. doi: 28663451.

Cemin, H. S, M. D. Tokach, S. S. Dritz, J. C. Woodworth, J. M. DeRouchey, and R. D Goodband. 2019. Meta-regression analysis to predict the influence of branched-chain and large neutral amino acids on growth performance of pigs. J. Anim. Sci. 97(6):2505-2514. doi:10.1093/jas/skz118.

Cemin, H. S., M. D. Tokach, J. C. Woodworth, S. S. Dritz, J. M. DeRouchey, and R. D. Goodband. 2019. Branched-chain amino acid interactions in growing pig diets. Transl. Anim. Sci. 3(4):1246-1253. doi:10.1093/tas/txz087.

Clizer, D. A. 2021. Evaluating impacts of tryptophan and branched chain amino acids in swine diets containing corn based dried distillers grains on the growth performance and carcass characteristics of grow-finish pigs. Ph.D. Theses and Dissertations. 5699. Available at: https://www.proquest.com/docview/2577533883?pq-origsite=gscholar&fromopenview=true

Cota, D., K. Proulx, K. A. Smith, S. C. Kozma, G. Thomas, S. C. Woods, and R. J. Seeley. 2006. Hypothalamic mtor signaling regulates food intake. Science. 312:927–930. doi:10.1126/science.1124147.

Fernstrom, J. D. 2013. Large neutral amino acids: dietary effects on brain neurochemistry and function. Amino Acids. 45:419–430. doi:10.1007/s00726-012-1330-y.

Harris, R. A., M. Joshi, N. H. Jeoung, and M. Obayashi. 2005. Overview of the molecular and biochemical basis of branched-chain amino acid catabolism. J. Nutr. 135(6 Suppl):1527S–1530S. doi:10.1093/jn/135.6.1527S.

Harper, A. E., R. H. Miller, and K. P. Block. 1984. Branched-chain amino acid metabolism. Annu. Rev. Nutr. 4:409–454. doi:10.1146/annurev.nu.04.070184.002205.

Henry, Y., B. Sève, Y. Colléaux, P. Ganier, C. Saligaut, and P. Jégo. 1992. Interactive effects of dietary levels of tryptophan and protein on voluntary feed intake and growth performance in pigs, in relation to plasma free amino acids and hypothalamic serotonin. J. Anim. Sci. 70:1873–1887. doi:10.2527/1992.7061873x.

Holen, J. P., R. D. Goodband, M. D. Tokach, J. C. Woodworth, J. M. DeRouchey, and J. T. Gebhardt. 2023. Effects of increasing soybean meal in corn-based diets on the growth

performance of late finishing pigs. Transl. Anim. Sci. 7(1): txac165. doi:10.1093/tas/txac165.

Kerkaert, H. R., H. S. Cemin, J. C. Woodworth, J. M. DeRouchey, S. S. Dritz, M. D. Tokach, R. D. Goodband, K. D. Haydon, C. W. Hastad, and Z. B. Post. 2021. Improving performance of finishing pigs with added valine, isoleucine, and tryptophan: validating a meta-analysis model, J. Anim. Sci. 99:1. doi:10.1093/jas/skab006.

Kwon, W. B., J. A. Soto, and H. H. Stein. 2022. Effects of dietary leucine and tryptophan on serotonin metabolism and growth performance of growing pigs. J. Anim. Sci. 100(1):skab356. doi:10.1093/jas/skab356.

Lee, J. H., J. H. Kim, J. D. Kim, S. W. Kim, and I. K. Han. 2001. Effects of low crude protein diets supplemented with synthetic amino acids on performance, nutrient utilization and carcass characteristics in finishing pigs reared using a phase feeding regimen. Asian-Australas. J. Anim. Sci. 14(5):655-667. Available at: https://www.animbiosci.org/upload/pdf/14-95.pdf

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

Pardridge, W. M. 1977. Kinetics of competitive inhibition of neutral amino acid transport across the blood-brain barrier. J. Neurochem. 28:103–108. doi:10.1111/j.1471-4159.1977.tb07714.x.

Soto, J. A., M. D. Tokach, S. S. Dritz, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, and F. Wu. 2019. Optimal dietary standardized ileal digestible lysine and crude protein concentration for growth and carcass performance in finishing pigs weighing greater than 100 kg. J. Anim. Sci. 97(4):1701-1711. doi:10.1093/jas/skz052.

Soto Gonzalez, J. A. 2018. Effects of low crude protein, amino acid fortified diets and neutral detergent fiber on finishing pig performance (Doctoral dissertation, Kansas State University). Available at: https://krex.k-state.edu/handle/2097/38578

Stein, H. H., M. L. Gibson, C. Pedersen, and M. G. Boersma. 2006. Amino acid and energy digestibility in ten samples of distillers dried grain with solubles fed to growing pigs. J. Anim. Sci. 84(4):853-860. doi:10.2527/2006.844853x.

Wessels, A. G., H. Kluge, F. Hirche, A. Kiowski, J. Bartelt, E. Corrent, and G. I. Stangl. 2016. High leucine intake reduces the concentration of hypothalamic serotonin in piglets. J. Anim. Sci. 94(3):26-29. doi:10.2527/jas.2015-9728.

Wessels, A. G., H. Kluge, F. Hirche, A. Kiowski, J. Bartelt, E. Corrent, and G. I. Stangl. 2016. 2016. High leucine diets stimulate cerebral branched-chain amino acid degradation and modify serotonin and ketone body concentrations in a pig model. PLoS ONE. 11(3):e0150376. doi:10.1371/journal.pone.0150376.

Whitney, M. H., G. C. Shurson, L. J. Johnston, D. M. Wulf, B. C. Shanks. 2006. Growth performance and carcass characteristics of grower-finisher pigs fed high-quality corn

distillers dried grain with solubles originating from a modern Midwestern ethanol plant, J. Anim. Sci. 84(12):3356–3363. doi:10.2527/jas.2006-099.

Table 1.1. Ingredient analysis, Exp.1 (as-fed basis)[1]

	Corn		SBM[2]		DDGS[3]	
Item, %	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
CP	7.56	8.17	45.80	46.36	29.03	29.77
Alanine	0.56	0.58	2.04	2.02	2.06	1.96
Arginine	0.38	0.37	3.42	3.18	1.33	1.32
Aspartic Acid	0.53	0.54	5.32	5.18	1.98	1.87
Cysteine	0.15	0.16	0.62	0.59	0.53	0.55
Glutamic Acid	1.36	1.47	8.07	8.45	5.11	5.04
Glycine	0.30	0.31	1.93	1.90	1.15	1.42
Histidine	0.21	0.20	1.22	1.15	0.81	0.75
Isoleucine	0.27	0.25	2.19	1.81	1.12	0.98
Leucine	0.88	0.90	3.54	3.37	3.36	3.22
Lysine	0.28	0.28	3.05	2.78	1.07	0.94
Methionine	0.17	0.17	0.71	0.57	0.60	0.54
Met + Cys	0.32	0.33	1.33	1.15	1.13	1.09
Phenylalanine	0.36	0.31	2.56	2.33	1.55	1.39
Proline	0.64	0.51	2.44	1.07	2.47	2.08
Serine	0.38	0.39	2.42	2.38	1.47	1.43
Threonine	0.28	0.28	1.90	1.80	1.14	1.04
Tyrosine	0.12	0.05	1.41	1.32	1.03	0.99
Valine	0.37	0.35	2.25	1.86	1.44	1.24
Tryptophan	0.06	0.07	0.68	0.62	0.25	0.25

[1]1 sample from each ingredient from each group was sent and analyzed in duplicate at Ajinomoto Laboratories, Eddyville, IA.
[2]Soybean meal
[3]Dried distillers grain with solubles

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

Val:Lys and Trp:Lys:	Standard			High		
Item, % SBM level:	Low	Medium	High	Low	Medium	High
Corn	65.85	62.15	58.45	65.60	62.00	58.35
Soybean meal	5.15	9.10	13.10	5.15	9.10	13.10
Corn DDGS, 7.5% oil	25.00	25.00	25.00	25.00	25.00	25.00
Choice white grease	1.00	1.10	1.10	1.00	1.10	1.10
Calcium carbonate	1.10	1.10	1.10	1.10	1.10	1.10
Monocalcium phosphate	0.35	0.30	0.30	0.35	0.30	0.30
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50
L-Lys HCl	0.54	0.41	0.29	0.54	0.41	0.29
DL-Met	0.04	0.02	--	0.04	0.02	--
L-Thr	0.10	0.10	0.02	0.10	0.10	0.02
L-Trp	0.06	0.03	0.01	0.10	0.07	0.05
L-Val	0.02	0.01	--	0.20	0.12	0.04
L-Ile	0.05	0.02	--	0.05	0.02	--
Vitamin premix[2]	0.13	0.13	0.13	0.13	0.13	0.13
Trace mineral premix[3]	0.13	0.13	0.13	0.13	0.13	0.13
Total, %	100	100	100	100	100	100
Calculated analysis						
Standard ileal digestible (SID) amino acids, %						
Lys	0.85	0.85	0.85	0.85	0.85	0.85
Ile:Lys	60	65	71	60	65	71
Leu:Lys	167	177	188	166	177	188
Met:Lys	34	34	35	34	34	35
Met and Cys:Lys	60	62	64	60	62	64
Thr:Lys	65	65	65	65	65	65
Trp:Lys	19.0	19.0	19.1	23.6	23.6	23.7
Val:Lys	70	77	83	87	87	88
His:Lys	41	45	49	41	45	49
Total Lys, %	1.01	1.03	1.04	1.01	1.03	1.04
NE, kcal/kg	2,588	2,590	2,590	2,590	2,593	2,593
SID Lys:NE, g/Mcal	3.28	3.28	3.28	3.28	3.28	3.28
Crude protein, %	15.8	17.0	18.3	15.9	17.1	18.4
Ca, %	0.59	0.59	0.59	0.59	0.59	0.59
P, %	0.47	0.47	0.48	0.47	0.47	0.48
STTD P, %	0.42	0.42	0.42	0.42	0.42	0.42

[1]Phase 1 was fed from approximately 63 to 82 kg. Nutrient loading values considered analyzed AA analysis (Ajinomoto Laboratories, Eddyville, IA) for the corn, SBM, and DDGS used in diets.

[2]Provided per kg of diet: 2067 IU vitamin A; 827 IU vitamin D3; 22.05 IU vitamin E; 24.80 mg niacin; 13.78 mg pantothenic acid; 1.65 mg vitamin K; 4.13 mg riboflavin; 0.02 mg vitamin B12; Ronozyme HiPhos GT 20,000 Phytase 625 FYT/kg.
[3]Provided per kg of diet: 2.27% CA; 13.75 ppm Cu; 0.25 ppm Iodine; 91.77 ppm Fe; 27.56 ppm Mn; 0.25 ppm Se; 91.77 ppm Zn. All trace minerals in this premix are from inorganic sources.

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

Val:Lys and Trp:Lys:	Standard			High		
Item, % SBM level:	Low	Medium	High	Low	Medium	High
Corn	69.75	66.05	62.30	69.60	65.95	62.30
Soybean meal	1.70	5.65	9.60	1.70	5.65	9.60
Corn DDGS, 7.5% oil	25.00	25.00	25.00	25.00	25.00	25.00
Choice white grease	1.00	1.05	1.10	1.00	1.05	1.10
Calcium carbonate	1.03	1.01	1.00	1.03	1.01	1.00
Monocalcium phosphate	0.10	0.05	--	0.10	0.05	--
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50
L-Lys HCl	0.51	0.38	0.30	0.51	0.38	0.30
L-Thr	0.10	0.05	--	0.10	0.05	--
L-Trp	0.06	0.03	0.01	0.09	0.07	0.04
L-Val	--	--	--	0.14	0.07	--
L-Ile	0.04	0.02	--	0.04	0.02	--
Vitamin premix[2]	0.10	0.10	0.10	0.10	0.10	0.10
Trace mineral premix[3]	0.10	0.10	0.10	0.10	0.10	0.10
Total, %	100	100	100	100	100	100
Calculated analysis						
Standard ileal digestible (SID) amino acids, %						
Lys	0.74	0.74	0.74	0.74	0.74	0.74
Ile:Lys	60	67	73	60	67	73
Leu:Lys	181	194	206	181	194	206
Met:Lys	32	35	38	32	35	37
Met and Cys:Lys	61	65	70	61	65	70
Thr:Lys	65	66	66	65	66	66
Trp:Lys	19.2	19.1	19.0	23.8	23.6	23.3
Val:Lys	70	79	88	88	88	88
His:Lys	43	47	52	42	47	52
Total Lys, %	0.90	0.91	0.92	0.90	0.91	0.92
NE, kcal/kg	2,597	2,599	2,601	2,599	2,601	2,601
SID Lys:NE, g/Mcal	2.85	2.85	2.85	2.85	2.85	2.84
Crude protein, %	14.4	15.7	17.0	14.5	15.8	17.1
Ca, %	0.50	0.50	0.50	0.50	0.50	0.50
P, %	0.40	0.41	0.41	0.40	0.41	0.41
STTD P, %	0.35	0.35	0.35	0.35	0.35	0.35

[1]Phase 2 was fed from approximately 82 to 109 kg. Nutrient loading values considered analyzed AA analysis (Ajinomoto Laboratories, Eddyville, IA) for the corn, SBM, and DDGS used in diets.
[2]Provided per kg of diet: 1653 IU vitamin A; 661 IU vitamin D3; 17.64 IU vitamin E; 19.84 mg niacin; 11.02 mg pantothenic acid; 1.32 mg vitamin K; 3.31 mg riboflavin; 0.01 mg vitamin B12; Ronozyme HiPhos GT 20,000 Phytase 500 FYT/kg.

[3]Provided per kg of diet: 1.82 CA; 11.00 ppm Cu; 0.20 ppm Iodine; 73.41 ppm Fe; 22.05 ppm Mn; 0.20 ppm Se; 73.41 ppm Zn. All trace minerals in this premix are from inorganic sources.

Table 1.4. Diet composition (as-fed basis) Phase 3, Exp. 1[1]

Val:Lys and Trp:Lys:	Standard			High		
Item, % SBM level:	Low	Medium	High	Low	Medium	High
Corn	71.75	68.00	64.25	71.60	67.90	64.20
Soybean meal	--	3.95	7.90	--	3.95	7.90
Corn DDGS, 7.5% oil	25.00	25.00	25.00	25.00	25.00	25.00
Choice white grease	1.00	1.05	1.10	1.00	1.05	1.10
Calcium carbonate	0.90	0.90	0.90	0.90	0.90	0.90
Monocalcium phosphate	0.10	0.05	--	0.10	0.05	--
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50
L-Lys HCl	0.45	0.32	0.20	0.45	0.32	0.20
L-Thr	0.10	0.05	--	0.10	0.05	--
L-Trp	0.05	0.02	0.01	0.08	0.05	0.03
L-Val	--	--	--	0.14	0.07	--
L-Ile	0.02	0.01	--	0.02	0.01	--
Vitamin premix[2]	0.08	0.08	0.08	0.08	0.08	0.08
Trace mineral premix[3]	0.08	0.08	0.08	0.08	0.08	0.08
Total, %	100	100	100	100	100	100
Calculated analysis						
Standard ileal digestible (SID) amino acids, %						
Lys	0.65	0.65	0.65	0.65	0.65	0.65
Ile:Lys	60	70	79	60	69	79
Leu:Lys	201	215	229	201	215	229
Met:Lys	35	38	41	35	38	41
Met and Cys:Lys	67	72	78	67	72	78
Thr:Lys	68	70	72	68	70	72
Trp:Lys	19.2	19.1	19.0	23.4	23.5	23.5
Val:Lys	76	86	96	96	96	96
His:Lys	46	52	57	46	52	57
Total Lys, %	0.80	0.81	0.82	0.80	0.81	0.82
NE, kcal/kg	2,599	2,601	2,604	2,604	2,604	2,604
SID Lys:NE, g/Mcal	2.50	2.50	2.50	2.50	2.50	2.50
Crude protein, %	13.7	15.0	16.4	13.8	15.1	16.4
Ca, %	0.44	0.44	0.44	0.44	0.44	0.44
P, %	0.39	0.40	0.41	0.39	0.40	0.41
STTD P, %	0.31	0.31	0.31	0.31	0.31	0.31

[1]Phase 3 was fed from approximately 109 to 136 kg. Nutrient loading values considered analyzed AA analysis (Ajinomoto Laboratories, Eddyville, IA) for the corn, SBM, and DDGS used in diets.
[2]Provided per kg of diet: 1240 IU vitamin A; 496 IU vitamin D3; 13.23 IU vitamin E; 14.88 mg niacin; 8.27 mg pantothenic acid; 0.99 mg vitamin K; 2.48 mg riboflavin; 0.01 mg vitamin B12; Ronozyme HiPhos GT 20,000 Phytase 375 FYT/kg.

[3]Provided per kg of diet: 1.36% CA; 8.25 ppm Cu; 0.15 ppm Iodine; 55.06 ppm Fe; 16.53 ppm Mn; 0.15 ppm Se; 55.06 ppm Zn. All trace minerals in this premix are from inorganic sources.

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

SBM level:	High	Medium	Low	Low
Item, % AA adjustment:	No	No	Yes	No
Corn	61.03	44.24	49.15	49.38
Soybean meal	35.26	27.15	21.53	21.51
DDGS	--	25.00	25.00	25.00
Choice white grease	1.00	1.00	1.00	1.00
Monocalcium phosphate	0.50	--	0.05	0.05
Calcium carbonate	1.15	1.45	1.48	1.48
Sodium chloride	0.50	0.50	0.50	0.50
Liquid lysine, 55%	0.29	0.47	0.72	0.72
DL-Met	0.07	--	0.06	0.06
L-Trp	--	0.02	0.05	0.04
L-Val	--	--	0.10	--
L-Ile	--	--	0.10	--
Thr[2]	0.05	0.03	0.13	0.13
Phytase[3]	0.05	0.05	0.05	0.05
Vitamin-trace mineral premix[4]	0.10	0.10	0.10	0.10
Total, %	100	100	100	100
Calculated analysis				
Standard ileal digestible (SID) amino acids, %				
Lys	1.20	1.20	1.20	1.20
Ile:Lys	68	68	68	60
Leu:Lys	136	158	147	147
Met:Lys	31	29	31	31
Met and Cys:Lys	56	56	56	56
Thr:Lys	61	61	61	61
Trp:Lys	20.1	20.1	20.1	19.0
Val:Lys	73	77	77	69
His:Lys	44	46	41	41
Total Lys, %	1.35	1.41	1.40	1.40
NE, kcal/kg	2,657	2,533	2,535	2,531
SID Lys:NE, g/Mcal	4.52	4.74	4.73	4.74
STTD P, %	0.39	0.39	0.39	0.39
Chemical analysis, %				
DM	86.65	87.32	87.58	86.87
CP	21.90	22.70	21.70	20.80
Ca	0.55	0.73	0.78	0.60
P	0.38	0.48	0.48	0.42

[1]Phase 1 was fed from approximately 23 to 45 kg.
[2]Thr Pro; CJ America-Bio, Downers Grove, IL.

[3]Optiphos (Huevepharma, Sofia, Bulgaria) was included at 1,251 FTU/kg providing an estimated release of 0.15% STTD P.
[4]Provided per kg of diet: 5,292 IU vitamin A; 1,323 IU vitamin D; 26.46 IU vitamin E; 2.65 mg vitamin K; 0.02 mg vitamin B12; 49.60 mg niacin; 16.53 mg pantothenic acid; 4.96 mg riboflavin; 16.50 mg Cu; 0.30 mg iodine; 110.00 mg iron; 33.10 mg manganese; 0.30 mg selenium; 110.00 mg zinc.

Table 1.6. Diet composition (as-fed basis) Phase 2, Exp. 2[1]

SBM level	High	Medium	Low	Low
Item, % AA adjustment:	No	No	Yes	No
Corn	69.66	52.39	80.37	79.23
Soybean meal	26.68	19.07	17.24	17.32
DDGS	--	25.00	25.00	25.00
Choice white grease	1.00	1.00	1.00	1.00
Monocalcium phosphate	0.55	0.05	0.10	0.10
Calcium carbonate	1.08	1.38	1.40	1.40
Sodium chloride	0.05	0.05	0.05	0.05
Liquid lysine, 55%	0.29	0.44	0.66	0.66
DL-Met	0.05	--	0.02	0.02
L-Trp	--	0.02	0.05	0.04
L-Val	--	--	0.08	--
L-Ile	--	--	0.08	--
Thr[2]	0.05	0.01	0.10	0.10
Phytase [3]	0.05	0.05	0.05	0.05
Vitamin-trace mineral premix[4]	0.10	0.10	0.10	0.10
Total, %	100	100	100	100
Calculated analysis				
Standard ileal digestible (SID) amino acids, %				
Lys	0.99	0.99	0.99	0.99
Ile:Lys	68	68	68	60
Leu:Lys	145	172	160	161
Met:Lys	32	31	30	30
Met and Cys:Lys	58	61	58	58
Thr:Lys	62	62	62	62
Trp:Lys	19.6	19.5	19.8	18.8
Val:Lys	74	80	80	72
His:Lys	45	48	43	43
Total Lys, %	1.12	1.18	1.17	1.17
NE, kcal/kg	2,657	2,533	2,535	2,531
SID Lys:NE, g/Mcal	3.73	3.91	3.91	3.91
STTD P, %	0.38	0.38	0.38	0.38
Chemical analysis, %				
DM	86.65	87.39	87.50	87.68
CP	20.80	22.70	20.70	21.60
Ca	0.59	0.50	0.63	0.73
P	0.38	0.48	0.46	0.47

[1]Phase 2 was fed from approximately 45 to 73 kg.
[2]Thr Pro; CJ America-Bio, Downers Grove, IL.

[3]Optiphos (Huevepharma, Sofia, Bulgaria) was included at 1,251 FTU/kg providing an estimated release of 0.15% STTD P.
[4]Provided per kg of diet: 5,292 IU vitamin A; 1,323 IU vitamin D; 26.46 IU vitamin E; 2.65 mg vitamin K; 0.02 mg vitamin B12; 49.60 mg niacin; 16.53 mg pantothenic acid; 4.96 mg riboflavin; 16.50 mg Cu; 0.30 mg iodine; 110.00 mg iron; 33.10 mg manganese; 0.30 mg selenium; 110.00 mg zinc.

Table 1.7 Diet composition (as-fed basis) Phase 3, Exp. 2[1]

SBM level	High	Medium	Low	Low
Item, % AA adjustment:	No	No	Yes	No
Corn	75.68	62.23	65.89	66.08
Soybean meal	20.93	14.46	10.27	10.26
DDGS	--	20.00	20.00	20.00
Choice white grease	1.00	1.00	1.00	1.00
Monocalcium phosphate	0.45	--	0.05	0.05
Calcium carbonate	0.98	1.25	1.28	1.28
Sodium chloride	0.50	0.50	0.50	0.50
Liquid lysine, 55%	0.25	0.39	0.58	0.58
DL-Met	0.03	--	--	--
L-Trp	0.02	0.03	0.06	0.03
L-Val	--	--	0.08	--
L-Ile	--	--	0.08	--
Thr[2]	0.05	0.02	0.10	0.10
Phytase[3]	0.03	0.03	0.03	0.03
Vitamin-trace mineral premix[4]	0.10	0.10	0.10	0.10
Total, %	100	100	100	100
Calculated analysis				
Standard ileal digestible (SID) amino acids, %				
Lys	0.83	0.83	0.83	0.83
Ile:Lys	69	69	69	60
Leu:Lys	157	182	170	170
Met:Lys	32	33	30	31
Met and Cys:Lys	60	64	60	60
Thr:Lys	64	64	64	64
Trp:Lys	21.3	21.4	21.5	18.6
Val:Lys	77	82	82	74
His:Lys	47	49	45	45
Total Lys, %	0.95	0.99	0.98	0.98
NE, kcal/kg	2,661	2,564	2,566	2,562
SID Lys:NE, g/Mcal	3.12	3.24	3.24	3.24
STTD P, %	0.32	0.32	0.32	0.32
Chemical analysis, %				
DM	85.14	85.45	85.40	85.17
CP	16.10	19.70	19.00	18.90
Ca	0.53	0.57	0.56	0.64
P	0.42	0.45	0.42	0.43

[1]Phase 3 was fed from approximately 73 to 100 kg.
[2]Thr Pro; CJ America-Bio, Downers Grove, IL.

[3]Optiphos (Huevepharma, Sofia, Bulgaria) was included at 626 FTU/kg providing an estimated release of 0.12 % STTD P.

[4]Provided per kg of diet: 5,292 IU vitamin A; 1,323 IU vitamin D; 26.46 IU vitamin E; 2.65 mg vitamin K; 0.02 mg vitamin B12; 49.60 mg niacin; 16.53 mg pantothenic acid; 4.96 mg riboflavin; 16.50 mg Cu; 0.30 mg iodine; 110.00 mg iron; 33.10 mg manganese; 0.30 mg selenium; 110.00 mg zinc.

Table 1.8. Diet composition (as-fed basis) Phase 4, Exp. 2[1]

Item, %	SBM level AA adjustment:	High No	Medium No	Low Yes	Low No
Corn		77.65	67.00	72.92	73.19
Soybean meal		19.63	14.96	8.36	8.34
DDGS		--	15.00	15.00	15.00
Choice white grease		1.00	1.00	1.00	1.00
Monocalcium phosphate		0.35	--	0.10	0.13
Calcium carbonate		0.93	1.15	1.15	1.15
Sodium chloride		0.50	0.50	0.50	0.50
Liquid lysine, 55%		0.14	0.24	0.54	0.54
DL-Met		--	--	0.02	0.02
L-Trp		--	0.01	0.05	0.03
L-Val		0.03	--	0.11	--
L-Ile		0.14	--	0.12	--
Thr[2]		0.03	0.01	0.12	0.12
Phytase[3]		0.03	0.03	0.03	0.03
Vitamin-trace mineral premix[4]		0.10	0.10	0.10	0.10
Total, %		100	100	100	100
Calculated analysis					
Standard ileal digestible (SID) amino acids, %					
Lys		0.74	0.74	0.74	0.74
Ile:Lys		75	75	75	60
Leu:Lys		172	194	172	172
Met:Lys		31	35	33	33
Met and Cys:Lys		63	69	63	63
Thr:Lys		67	67	67	67
Trp:Lys		21.0	21.0	21.3	18.7
Val:Lys		84	88	88	74
His:Lys		51	54	45	45
Total Lys, %		0.85	0.89	0.87	0.87
NE, kcal/kg		2,665	2,593	2,463	2,588
SID Lys:NE, g/Mcal		2.78	2.85	2.85	2.86
STTD P, %		0.30	0.30	0.30	0.29
Chemical analysis, %					
DM		86.28	85.88	86.00	85.48
CP		12.50	17.80	13.80	14.50
Ca		0.58	0.62	0.56	0.59
P		0.36	0.34	0.33	0.36

[1]Phase 4 was fed from approximately 100 to 136 kg.

[2]Thr Pro; CJ America-Bio, Downers Grove, IL.
[3]Optiphos (Huevepharma, Sofia, Bulgaria) was included at 626 FTU/kg providing an estimated release of 0.12 % STTD P.
[4]Provided per kg of diet: 5,292 IU vitamin A; 1,323 IU vitamin D; 26.46 IU vitamin E; 2.65 mg vitamin K; 0.02 mg vitamin B12; 49.60 mg niacin; 16.53 mg pantothenic acid; 4.96 mg riboflavin; 16.50 mg Cu; 0.30 mg iodine; 110.00 mg iron; 33.10 mg manganese; 0.30 mg selenium; 110.00 mg zinc.

Table 1.9. Effects of Increasing Soybean Meal and Val:Lys and Trp:Lys Ratios in diets containing DDGS on finishing pig performance, Exp.1[1]

Val:Lys and Trp:Lys:	Standard			High				*P* =		
								SBM level		AA
Item SBM level:	Low	Medium	High	Low	Medium	High	SEM	Linear	Quadratic	ratio
BW, kg										
d 0	62.9	62.9	62.8	62.9	62.9	63.0	0.739	0.936	0.947	0.832
d 19	84.1	84.9	84.6	84.1	84.2	84.9	0.923	0.236	0.794	0.802
d 48	115.4	116.5	116.3	115.7	115.7	116.9	1.016	0.144	0.969	0.939
d 61	129.7	132.4	131.1	131.3	131.4	132.3	1.464	0.192	0.315	0.390
Phase 1 (d 0 to 19)										
ADG, kg	1.04	1.07	1.06	1.04	1.04	1.07	0.020	0.162	0.838	0.762
ADFI, kg	2.59	2.66	2.59	2.59	2.66	2.69	0.040	0.172	0.164	0.244
G:F	0.402	0.402	0.411	0.402	0.392	0.398	0.006	0.739	0.236	0.116
Phase 2 (d 19-48)										
ADG, kg	1.10	1.11	1.10	1.11	1.11	1.12	0.015	0.484	0.952	0.524
ADFI, kg	3.12	3.21	3.15	3.13	3.18	3.21	0.042	0.129	0.185	0.734
G:F	0.353	0.345	0.351	0.354	0.349	0.350	0.004	0.339	0.110	0.683
Phase 3 (d 48-61)										
ADG, kg[2]	0.87	0.96	0.87	0.92	0.93	0.90	0.029	0.760	0.009	0.254
ADFI, kg	3.08	3.16	3.11	3.08	3.13	3.18	0.056	0.133	0.375	0.661
G:F	0.281	0.302	0.281	0.299	0.297	0.284	0.006	0.192	0.007	0.244
Overall (d 0 to 61)										
ADG, kg	1.02	1.05	1.03	1.04	1.04	1.05	0.015	0.281	0.260	0.413
ADFI, kg	2.94	3.02	2.96	2.94	3.00	3.04	0.039	0.103	0.187	0.509
G:F[3]	0.346	0.348	0.348	0.352	0.347	0.345	0.003	0.389	0.832	0.746

[abc] Means within row with different superscripts differ ($P < 0.05$)

[1] A total of 626 pigs (initial BW of 62.6 ± 0.74 kg) were used in 2 groups in a 61-d finisher trial with 8-9 pigs per pen and 12 pens per treatment. SBM × AA ratio interactions ($P > 0.10$) unless otherwise specified.

[2] SBM × AA ratio, $P = 0.084$. Quadratic SBM within standard Val:Lys and Trp:Lys, $P = 0.003$; Quadratic within increased Val:Lys and Trp:Lys, $P = 0.501$.

[3] SBM × AA ratio, $P = 0.052$. Linear SBM within standard Val:Lys and Trp:Lys, $P = 0.435$; Linear within increased Val:Lys and Trp:Lys, $P = 0.049$.

Table 1.10. Comparison of predicted performance characteristics based on Cemin et al. (2019) model to actual observed data, Exp.1

Val:Lys and Trp:Lys:	Standard			High		
Item SBM level:	Low	Medium	High	Low	Medium	High
Overall						
Predicted						
ADG, kg	1.02	1.04	1.04	1.08	1.09	1.08
ADFI, kg	2.94	2.96	2.94	3.06	3.07	3.04
G:F	0.346	0.350	0.354	0.354	0.354	0.354
Actual						
ADG, kg	1.02	1.05	1.03	1.04	1.04	1.05
ADFI, kg	2.94	3.02	2.96	2.94	3.00	3.04
G:F	0.346	0.348	0.348	0.352	0.347	0.345
% of predicted						
ADG, kg	100	102	99	96	96	98
ADFI, kg	100	102	101	96	98	100
G:F	100	99	98	99	98	97

Table 1.11. Effects of SBM levels and AA adjustment in diets with or without DDGS on finishing pig performance and carcass characteristics, Exp. 2[1]

SBM Level[2]	High	Medium	Low	Low			
AA Adjustment[3]	No	No	Yes	No		*P* =	
DDGS[4]	No	Yes	Yes	Yes	SEM	Treatment	Control vs. DDGS[5]
BW, kg							
d 0	26.5	26.5	26.5	26.5	0.57	0.999	0.977
d 56	77.4[a]	74.3[ab]	74.6[ab]	73.6[b]	1.00	0.050	0.007
d 121	138.7	135.0	137.3	135.1	1.20	0.100	0.044
Grower (d 0-56)							
ADG, kg	0.91[a]	0.85[b]	0.84[b]	0.83[b]	0.013	0.001	< 0.001
ADFI, kg	1.95	1.89	1.88	1.89	0.027	0.264	0.050
G:F	0.465[a]	0.448[b]	0.448[b]	0.442[b]	0.004	0.001	< 0.001
Finisher (d 56-121)							
ADG, kg	0.97	0.96	0.99	0.98	0.011	0.189	0.409
ADFI, kg	3.12	3.13	3.20	3.21	0.033	0.167	0.143
G:F	0.310	0.307	0.311	0.306	0.003	0.717	0.586
Overall (d 0 to 121)							
ADG, kg	0.94	0.91	0.92	0.91	0.009	0.060	0.014
ADFI, kg	2.56	2.53	2.57	2.57	0.026	0.716	0.859
G:F	0.367[a]	0.358[ab]	0.360[ab]	0.355[b]	0.003	0.031	0.007
Carcass Characteristics[6]							
HCW, kg	103.4	100.2	101.6	100.5	2.03	0.074	0.018
Carcass yield, %	73.3	73.0	73.2	73.1	0.003	0.940	0.639
Lean, %	56.24	56.34	56.03	56.19	0.222	0.788	0.853
Loin depth, cm.	6.68	6.55	6.50	6.60	0.025	0.187	0.066
Back fat depth, cm.	1.75	1.70	1.75	1.73	0.012	0.749	0.584

[a-c]Means within row with different superscripts differ ($P < 0.05$).

[1]A total of 1,080 pigs (initial BW 26.5 ± 0.57 kg) were used in in a 121-d finisher trial with 27 pigs per pen and 10 pens per treatment.

[2]SBM levels ranged from 21.51 (low) to 35.26% (high) in phase 1, 17.24 to 26.68% in phase 2, 10.26 to 20.93% in phase 3, and 8.34 to 19.63% in phase 4.

[3] The AA adjustment more specifically included adding additional Val, Ile and Trp so that the individual AA ratios for Val:Lys, Ile:Lys, and Trp:Lys within treatment 3 (DDGS + low SBM) matched that of the medium SBM diet and the Ile+Val+Trp:Leu ratio was equal to that of the high SBM diet.

[4]DDGS included in medium and low SBM diets at 25% in phases 1 and 2, 20% in phase 3, and 15% in phase 4.

[5]Contrast comparing the response of pigs fed diets without DDGS compared to the mean of the pigs fed diets with DDGS.

[6]In the analysis for backfat depth, lean, and loin, HCW was used as a covariate in the model.

Table 1.12. Comparison of predicted performance characteristics based on Cemin et al. (2019) model to actual observed data, Exp.2

SBM Level[2]	High	Medium	Low	Low
AA Adjustment[3]	No	No	Yes	No
DDGS[4]	No	Yes	Yes	Yes
Overall				
Predicted				
ADG, kg	0.94	0.93	0.94	0.90
ADFI, kg	2.56	2.53	2.55	2.50
G:F	0.367	0.368	0.370	0.359
Actual				
ADG, kg	0.94	0.91	0.92	0.91
ADFI, kg	2.56	2.53	2.57	2.57
G:F	0.367	0.358	0.360	0.355
% of predicted				
ADG, kg	100	97	98	101
ADFI, kg	100	100	100	102
G:F	100	97	97	99

Chapter 2 - Evaluating HiPhorius phytase on finishing pig growth performance, serum chemistry, bone mineralization, and carcass characteristics

Abstract

Two experiments were conducted to determine the effect of HiPhorius (DSM Nutritional Products, Parsippany, NJ) phytase on finishing pig growth performance, serum chemistry, bone mineralization, and carcass characteristics. In Exp. 1, 1,161 pigs (PIC 337 × 1050, initially 36.7 ± 0.48 kg) were used in a 105-d trial. There were 27 pigs per pen and 10 or 11 replications per treatment**.** Treatments consisted of: 1) Control diet with no added phytase and formulated to NRC (2012) requirement estimates for standard total tract digestible (STTD) P; 2) 600 FYT/kg added phytase formulated to the same STTD P as the control diet considering a release of 0.13% STTD P and 0.095% STTD Ca; 3) 1,000 FYT/kg added phytase formulated to the same STTD P as the control diet considering release of 0.16% STTD P and 0.107% STTD Ca and 4) high STTD P (no phytase; approximately 22% above NRC requirement estimates). All diets were formulated to a 1.30:1 STTD Ca:STTD P ratio. Overall, pigs fed NRC (2012) or high STTD P had increased ADG ($P < 0.05$) compared to pigs fed the treatments with added phytase. Pigs fed diets with phytase tended to have decreased ($P = 0.056$) 25-hydroxyvitamin-D3 compared to pigs fed NRC levels of STTD P. In Exp. 2, 1,160 pigs (PIC 337 × 1050, initially 75.9 ± 1.32 kg) were used in a 58-d trial. There were 27 pigs per pen and 11 replications per treatment**.** Treatments were the same as in Exp.1 except diets were formulated to the same total Ca:P ratio (P1: 1.15:1; P2: 1.12:1) without a STTD Ca release consideration from phytase. Overall, there were no differences in ADG, ADFI or G:F among treatments ($P > 0.10$). For pigs fed NRC or

high STTD P, there was an increase ($P < 0.05$) in metacarpal bone density, and a tendency for increased bone ash weight (g) ($P < 0.05$) and percentage bone ash ($P < 0.10$) compared to pigs fed treatments containing phytase**.** In conclusion, regardless of diet formulation strategy, decreased growth performance (Exp.1) and bone mineralization (Exp.2) was observed, indicating that the phytase release provided by the phytase was less than the assumed release used in diet formulation.

Key Words: bone ash, bone density, calcium, finishing pigs, phosphorus, phytase

List of abbreviations

ADG, average daily gain

ADFI, average daily feed intake

aP, available phosphorus

BW, body weight

Ca:P, calcium-to-phosphorus ratio

FYT, phytase unit

G:F, gain-to-feed

NE, net energy

SID, standardized ileal digestibility

STTD, standard total tract digestibility

STTD Ca: STTD P, standard total tract digestible calcium-to-phosphorus ratio

Introduction

Phytase is an enzyme commonly added to swine diets to improve the digestibility of phytate-bound-phosphorus (Rutherford et al., 2014). Increasing legislation designed to reduce P excretion has contributed to the growing acceptance of phytase as it lessens P excretion, allows for less expensive diets, and delays the depletion of nonrenewable global P reserves (Mullaney et al., 2000; Selle and Ravindran 2008).

Exogenous phytase has also been shown to improve Ca digestibility (Selle et al., 2009). Partial enzymatic hydrolysis of phytate by exogenous phytase reduces the extent of Ca–phytate complex formation which increases the availability of Ca and phytate-bound P (Selle et al., 2009). Because of the improved digestibility of Ca by phytase, most literature agrees that relatively low dietary Ca levels and 'narrow' Ca:P ratios should be used in pig diets containing exogenous phytase (Lei et al. 1994; Zeng et al., 2011; Becker et al., 2020). However, the industry continues to shift to formulating diets using STTD Ca:STTD P ratios rather than on a total Ca:P basis. Furthermore, Lagos et al. (2019) indicates that using total Ca and P may limit the Ca required to maximize bone mineral content, and suggested that a narrow STTD Ca to STTD P ratio is needed (1.23:1) to assure adequate bone mineralization without affecting growth performance. Thus, two different diet strategies were utilized in the current study, testing the effect of the phytase when using consistent total and digestible Ca:P ratios.

There are many commercially available phytase sources for producers to consider; however, not all phytase sources provide the same benefit when included at similar rates (Igbasan et al., 2000; Gonçalves et al, 2016). Thus, when a new phytase enters the marketplace, feeding studies are required to validate its efficacy. HiPhorius phytase is a new source of phytase (DSM Nutritional Products, Parsippany, NJ) and is a modified *C. braakii* phytase expressed

in *A. oryzae*. In recent studies, HiPhorius phytase improved growth performance and bone mineralization when fed to nursery pigs (Thorsen et al., 2021; Zhai et al., 2023a). However, no studies currently test this phytase source in finishing pigs.

The main questions in each of the experiments was 1) whether replacing monocalcium P and limestone with phytase would release P sufficient to demonstrate similar performance to that of pigs fed diets formulated to NRC levels of STTD P without phytase, and 2) if the STTD P requirement was greater than suggested by the NRC (2012) and thus feeding high STTD P would result in improved performance and bone mineralization. Therefore the objective of these experiments was to determine performance, serum chemistry, carcass characteristics, and bone mineralization of finishing pigs fed various levels of a newly introduced phytase (HiPhorius) when diets were formulated either to the same STTD Ca:STTD P or Ca:P ratio. Based on diet formulation, it was hypothesized that the first three treatments (NRC STTD P, 600 and 1000 FYT/kg phytase) would have similar performance based on the release values given by the phytase. Additionally, based on Vier et al. (2019) we hypothesized that diets formulated to STTD P levels above the NRC (2012) requirement estimate would result in improved growth performance and bone mineralization.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in both these experiments (4375). Both experiments were conducted at a commercial research-finishing site in southwest Minnesota. The barn was mechanically ventilated and double-curtain-sided. Each pen was equipped with a 5-hole stainless steel dry self-feeder and a bowl waterer for *ad libitum* access to feed and water. Pigs were provided 0.62 m^2 of floor space per pig until 120 kg and then 0.73 m^2 until the remaining pigs were marketed. In both

studies, daily feed additions to each pen were accomplished using a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) able to record the amount of feed provided for individual pens. Pigs were weighed approximately every 14 d to determine ADG, average daily feed intake (ADFI), and gain-to-feed ratio (G:F).

Diet manufacturing

HiPhorius phytase was provided by DSM Nutritional Products, Parsippany, NJ. The phytase was analyzed and the concentration of the product was 2,402 FYT/g which was then used in diet formulation in both experiments (DSM Laboratory, Belvidere, NJ). All finishing diets were manufactured at the New Horizon Farms Feed Mill in Pipestone, MN.

All diets were formulated to meet or exceed NRC (2012) requirement estimates for all nutrients. Complete diet samples were collected in phases 1, 2, 3 of Exp.1 and phase 2 of Exp.2. Diet samples in phase 4 of Exp.1 and phase 1 of Exp.2 were not collected at the farm. Samples were stored at -20°C until they were submitted for analysis of DM and CP (Midwest Laboratories, Omaha, NE), and Ca and P (KSU Soils lab). Standard procedures from AOAC (2023) were followed for analysis of moisture (Method 930.15) and CP (Method 990.03). Ca and P samples were analyzed by an Inductively Coupled Plasma (ICP) Spectrometer (Model 5800 ICP OES Agilent Technologies, Varian Australia Pty Ltd, Mulgrave, Victoria Australia). In phase 1 and 3 of Exp.1, diets were analyzed to verify phytase additions (DSM Laboratory, Belvidere, NJ).

Experiment 1

A total of 1,161 pigs (PIC 337 × 1050, initially 36.7 ± 0.48 kg) were used in a 105-d growth trial. Pigs were randomly assigned to 1 of 4 dietary treatments in a completely randomized design. There were 27 pigs per pen and 10 or 11 replications per treatment**.** Dietary treatments consisted of: 1) a control diet without phytase, formulated to NRC (2012) requirement estimates for STTD P; 2) 600 FYT/kg formulated to the same STTD Ca and P as the control diet considering a release of 0.13% STTD P and 0.095% STTD Ca, 3) 1,000 FYT/kg formulated to the same STTD Ca and P as the control diet considering a release of 0.16% STTD P and 0.107% STTD Ca and 4) high STTD P (no phytase; approximately 22% above NRC 2012) based on the results of Vier et al. (2019) indicating improved growth performance when pigs were fed STTD P levels above the NRC (2012) requirement estimates. All diets were formulated to a 1.30:1 STTD Ca:STTD P ratio considering the release provided by phytase. Dietary treatments were fed in 4 phases (Tables 1 to 4). Phase 1 was fed from 27 to 50 kg, phase 2 from 50 to 75 kg, phase 3 from 75 to 100 kg, and phase 4 from 100 to approximately 132 kg.

Blood was collected from one pig per pen approximately 12 h prior to harvest into vacutainer tubes that did not contain anticoagulant. Each blood sample was centrifuged for 10 min at 3400 rpm. Serum samples were immediately placed on dry ice and shipped to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) in Ames, IA. Analysis included serum chemistry, 25-hydroxyvitamin-D3, and 1,25-dihydroxyvitamin-D3 at the ISU-VDL and Heartland Assays, respectively (Ames, IA). For 1,25-dihydroxyvitamin-D3, samples, control, and standards were spiked with a d-1,25-dihydroxyvitamin-D3 internal standard and then protein precipitated. The samples and controls were purified by liquid-liquid extraction followed by solid phase extraction. Samples, controls, and standards were then derivatized using 4-Phenly-

1,2,4-triazoline-3,5-dione followed by additional SPE clean-up. The samples and controls were reconstituted in LC/MS/MS mobile phase and injected into Agilent 1290 high pressure liquid chromatography system using a Zorbaz Eclipse Plus C-18 column coupled to 6460 Agilent triple tandem mass spectrometer (MS/MS) in positive ion mode using an ESI source. The standard curve ranged from 10.0 pg/mL to 1280.0 pg/mL in order to quantitate the levels in most biological fluids. The limit of detection was found to be 5.0 pg/mL for 1, 25-(OH)2D2/3 and the limit of quantitation was found to be 10.0 pg/mL Matrix spiked recoveries of 50.0 pg/mL were shown to average 90% 1, 25-(OH)2D2 (SEM of 10%) and 98% for 1, 25-(OH)2D3 (average SEM of 3.0%). In-house prepared control samples were made from certified reference material (CRM) with a value of 50.0 to 100.0 and 150.0 pg/mL and were assayed with an overall inter-assay coefficient of variation of 9.4% for 50.0 pg/mL and 4.3% for 150.0 pg/mL 1, 25-(OH)2D2/3. Intra-assay coefficient of variation was found to be 9.9% for 50.0 pg/mL, 6.1% for 100.0 pg/mL, 2.5% for 150.0 pg/mL 1, 25-(OH)2D2/3.

On d 88 the 3 heaviest pigs in each pen were selected and marketed. These pigs were included in growth performance data but not in the blood analysis or final pen carcass data. On the last day of the trial (d 105), final pen weights were obtained, and remaining pigs were tattooed with a pen identification number and transported to a U.S. Department of Agriculture-inspected packing plant (JBS Swift, Worthington, MN) for carcass data collection. Carcass measurements included hot carcass weight, loin depth, backfat depth, and percentage lean. Percentage lean was calculated from a plant proprietary equation. Carcass yield was calculated by dividing the pen average HCW by the pen average final live weight obtained at the farm.

Experiment 2

A total of 1,160 pigs (PIC 337 × 1050, initially 75.9 ± 1.32 kg) were used in a 58-d study. Pens were randomly assigned to 1 of 4 dietary treatments in a completely randomized design. There were approximately 27 pigs per pen and 11 replications per treatment**.** For this trial**,** treatments were formulated using a similar diet strategy to that of the first experiment, apart from slight differences in the monocalcium phosphate and limestone amounts so that the diets were formulated to the same total Ca:P ratio (Tables 5 and 6). Dietary treatments consisted of: 1) a control diet without added phytase formulated to NRC (2012) requirement for STTD P 2) 600 FYT/kg phytase formulated to the same STTD P as the control diet considering a release of 0.13% STTD P 3) 1,000 FYT/kg added phytase formulated to the same STTD P as the control diet considering a release of 0.16% STTD P, and 4) high levels for STTD P (no phytase; approximately 22% STTD P above NRC requirement estimates; Vier et al., 2019).

Blood was collected from one pig per pen approximately 12 h prior to harvest into blood tubes that did not contain anticoagulant. Each blood tube was spun down via a centrifuge for 10 min at 3400 rpm to obtain serum samples. Serum samples were immediately placed on dry ice and shipped to the ISU-VDL in Ames, IA. Analyses consisted of 25-hydroxyvitamin-D3. Along with blood collection, one pig per pen (visually identified as having an average body weight of the pen and with barrows and gilts equally represented within treatment) was transported to a commercial abattoir (JBS Swift, Worthington, MN) for collection of the right foot. The feet were then transported to Kansas State University and the third and fourth metacarpal bones were collected. From each foot, the third metacarpal was utilized to determine bone density and the fourth metacarpal for de-fatted bone ash (Wensley et al., 2020a). Bones were stored at -20°C until analysis. Briefly, bones were cleaned of extraneous soft tissue, and the weight of each bone

was recorded and then placed in ultrapure water and vacuumed for 4 h under a negative pressure vacuum at 1.06 kg per cubic centimeter. Bones were then submerged in water and weighed again. Archimedes principle was used to determine bone density; the weight or volume of the liquid was assumed equal to the weight of the initial bone minus the submerged bone weight. Taking the initial bone weight divided by the volume of the liquid we were able to determine bone density on each bone. For bone ash analysis, bones were cleaned of extraneous soft tissue, defatted in ether for 7 d, dried for 105℃ for 7 d in a drying oven, and then ashed in a muffle furnace at 600℃ for 24 h to determine total ash weight and percentage ash relative to dried bone weight (Wensley et al., 2020a). Calcium and P content within each bone was also determined (Williams et al., 2022). Between 0.025 and 0.040 g of bone ash from each bone was added to 100 uL nitric acid. Tubes were heated at 16°C for 6 h, diluted 1:10 with ultrapure water and then sent to the KSU Soils Lab (Manhattan, KS) where Ca and P quantity was measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

On d 45 the 3 heaviest pigs in each pen were selected and marketed. These pigs were included in growth performance data but not in the blood analysis or final pen carcass data. On the last day of the trial (d 58), final pen weights were obtained, and the remaining pigs were tattooed with a pen identification number and transported to a U.S. Department of Agriculture-inspected packing plant (JBS Swift, Worthington, MN) for carcass data collection using identical procedures to Exp. 1.

Statistical Analysis

Data were analyzed as a completely randomized design for one-way ANOVA using the lm function from the lme4 package in R (version 3.5.2 (2018-07-02) with pen as the

experimental unit, treatment considered fixed effect for all performance, blood, and bone criteria. For analysis of carcass data, individual carcass served as the observational unit and pen was included within the model as a random intercept to account for subsampling with multiple observations within each experimental unit. Lean percentage, loin depth, and backfat depth considered HCW as a covariate in the model. Means within a row with different superscripts differ (a, b, c, $P < 0.05$; x, y, z, $0.05 < P \leq 0.10$) using a Tukey multiple comparison adjustment. Results were considered significant with P-values ≤ 0.05 and were considered marginally significant with P-values > 0.05 and ≤ 0.10.

Results

Experiment 1

For the grower phase (d 0 to 54), there was evidence of a treatment effect for ADG (F-test: $P = 0.030$; Table 7) with dietary treatments formulated with phytase having a tendency for decreased ADG than pigs fed high levels of STTD P (T-test: $P \leq 0.066$) with pigs fed NRC STTD P intermediate. There was a tendency for a treatment effect of G:F ($P = 0.071$) with pigs fed current NRC (2012) requirement estimates for STTD P numerically having the greatest G:F compared to all other dietary treatments. For the finisher phase (d 54 to 105), no significant differences ($P > 0.10$) between treatments were observed for ADG, ADFI, or G:F.

Overall (d 0 to 105), pigs fed NRC or high STTD P had increased ADG ($P < 0.05$) compared to those pigs fed diets with either 600 or 1,000 FYT/kg phytase. Pigs fed 1,000 FYT/kg phytase had decreased G:F ($P < 0.05$) compared to pigs fed the NRC (2012) control diet, with pigs fed the other two treatments intermediate.

There was evidence of a difference in hot carcass weight between treatments ($P = 0.047$), but no evidence of pairwise differences between treatments ($P > 0.10$). There was also a

tendency ($P = 0.061$) for backfat, with pigs fed NRC levels of STTD P having lower backfat compared to all other treatments. For lean percentage, there was a statistical treatment difference ($P = 0.021$) with pigs fed the NRC (2012) control treatment having higher lean percentage than pigs fed either treatment with added phytase ($P < 0.05$), with pigs fed the high STTD P intermediate. No other carcass characteristics significantly differed between dietary treatments ($P > 0.10$).

There was a treatment effect for 25-hydroxyvitamin-D3 concentration ($P = 0.022$; Table 8). Pigs fed diets with Hiphorius phytase had lower 25-hydroxyvitamin-D3 than pigs fed NRC levels of STTD P ($P = 0.056$). There was no evidence of difference for all other blood criteria measured ($P > 0.10$) including minerals and 1,25-dihydroxyvitamin-D3.

Experiment 2

There were no statistical differences between dietary treatment for BW throughout the study ($P > 0.10$; Table 9). From d 0 to 17, pigs fed NRC levels of STTD P had increased ADG and ADFI ($P < 0.05$) compared to pigs fed to the same STTD P considering the release of 600 FYT Hiphorius phytase, with pigs fed either 1,000 FYT/kg Hiphorius or high STTD P intermediate. There were no statistical differences ($P > 0.10$) between dietary treatments on any performance criteria throughout the remaining finishing periods, overall, or carcass characteristics.

Metacarpals were denser ($P < 0.05$) for pigs fed either NRC (2012) or high levels of STTD P compared to pigs fed 600 FYT/kg phytase, with pigs fed 1,000 FYT/kg phytase intermediate (Table 10). There was also a tendency for a treatment effect ($P < 0.10$) for bone ash weight and percentage bone ash, where pigs fed NRC or high STTD P levels tended to have

greater ash weight and percentage bone ash compared to pigs fed treatments including phytase. No significant differences ($P > 0.10$) were observed for weight or concentration of Ca and P in metacarpal bone ash between treatments. Levels of 25-hydroxyvitamin-D3 in the blood were not statistically different between treatments ($P > 0.10$).

Discussion

Calcium and P play an integral role in growth and bone mineralization of pigs (O'Doherty et. al., 2010). The supplementation of phytase in low P diets in grower-finisher pigs has been shown to improve bone mineralization (O'Doherty et al., 2010). However, it has also been reported that maximum bone mineralization levels are achieved at higher P levels than are required for optimum gain and feed utilization (NRC, 2012). Vier et al. (2019) observed that a greater P requirement [122% of STTD-P requirements] for grow-finish pigs was required for maximum growth performance and bone mineralization than suggested by NRC (2012).

While growth and bone mineralization require both Ca and P, it is important to understand the interaction between the two minerals. As Ca increases in the diet, more P will be bound in undigestible Ca-P complexes in the small intestine and thereby reduce P availability (Lagos et al. 2019). Thus, if the diet meets the requirement for STTD P (NRC, 2012), a low concentration of STTD Ca may be beneficial for pig growth (Lagos et al. 2019; Gonzalez-Vega et al. 2016). Both minerals can influence the digestibility and availability of each other for growth and bone mineralization.

It is important to understand Ca:P ratios and how they differ on a total vs. STTD basis. Standard total tract digestible Ca:STTD P ratios consider the amount of each mineral that is digested by the pig, rather than simply the amount that is present in the diet. We used two

different approaches to diet formulation in the two experiments based on previous literature indicating potential differences in growth performance and bone mineralization based on formulation strategy (Lagos et al. 2019). In Exp. 1, diets were formulated to the same STTD Ca:STTD P ratio, while in Exp. 2, diets were formulated so that the Ca:P ratio on a total basis was the same among treatments.

There were several similarities observed between the two experiments regardless of differing formulation strategy. In Exp.1, diets were formulated to equal STTD Ca:STTD P ratios (1.30:1). In Exp.1, there was a reduction in ADG and G:F and 25-OH-D3 observed for pigs fed diets with phytase throughout the trial. This conflicted with our hypothesis and past literature testing other phytase sources included in finishing, where pig performance was maintained when replacing inorganic P with phytase in diets (Augspurger et al, 2007). With the unexpected results of this experiment, the second experiment was conducted using a slightly different diet formulation strategy. Zhai et al. (2023b) tested various Ca:P ratios in nursery pig diets with phytase and found that increasing Ca:P (0.58:1 to 1.30:1) negatively impacted growth performance. Liu et al. (1998) also observed that increasing Ca:P ratio (from 1.0:1 to 1.3:1 to 1.5:1) decreased growth performance and bone mineralization in grow-finish pigs. These authors speculated that the negative effects of wider Ca:P ratios could be due to decreased phytase efficacy due to decreased phytase activity (Qian et al., 1996), formation of insoluble phytate complex not accessible for hydrolysis by phytase (Selle et al., 2009), and/or an overestimation of P release by phytase (Olsen et al., 2021). Using this previous literature, diets in Exp. 2 were formulated so that total Ca:P ratios were fixed (1.12:1). However, in the first period of Exp. 2, pigs fed diets containing phytase also had decreased growth performance compared to pigs fed only inorganic P. While there was a numeric reduction in overall ADG for pigs fed 600 FYT/kg of phytase, it was not

statistically significant. Data from Exp. 2 also showed that pigs fed only inorganic sources of P had increased metacarpal bone density and percentage bone ash compared to pigs fed diets with phytase which might suggest that the STTD P release considered for the phytase was overestimated. Ultimately, based on diet formulation and claimed release values from the phytase at 600 and 1,000 FYT/kg, these inclusion levels did not appear to provide the expected level of STTD P release based on reduced growth performance and bone mineralization compared to pigs fed NRC levels of STTD P.

Several possible explanations could explain the results of these studies. Using loading values (Lee et al., 2021) for the phytate concentrations within the ingredients gives a phytate P value in the diets of 0.25 in phase 1, 0.23% in phase 2, and 0.22% in phases 3 and 4. There likely is a limit (~70-80%) to the amount of phytate-bound P that can be released by phytase (Espinosa et al., 2022; Lagos et al., 2022). Assuming only 70% of the phytate-bound P was able to be released by the phytase, this suggests that in phases 3 and 4 there was only 0.15% STTD P available to be released from phytate, and thus there was not enough substrate available to allow a 0.16% STTD P release for the 1,000 FYT/kg phytase diet. While it is possible for a 0.13% STTD P release for diets with 600 FYT/kg, the lack of substrate in diets in still a concern. Comparing the results of the two studies, the negative effects in growth performance were specifically seen earlier in finishing compared to later finishing. Monocalcium phosphate was completely removed in the later finishing diets that included phytase to formulate STTD P as low as possible assuming the release of P from phytase. Thus, in later finishing when no differences in performance were observed, the STTD P level was not able to be further lowered in those diets and likely remained adequate. Another key point that suggests a P deficiency in these trials is that in Exp. 1, lower 25-OH-D3 was observed in pigs fed either inclusion level of phytase. 25-

OH-D3 is converted to 1,25-OH-D3 which is used for Ca and P regulation. Epidemiological data have placed increased emphasis on monitoring blood levels of 25-OH-D3, the circulating precursor to 1,25-(OH)2D because higher blood 25-OH-D3 values correlate with a number of clinical parameters (e.g., increased bone mineral density and strength; Jones et al., 2007). The decrease in 25-OH-D3 in pigs fed treatments with phytase in Exp.1 equates to those pigs not converting as much 25-OH-D3 to 1,25-OH-D3 because additional Ca and P regulation is not as necessary compared to pigs with a P deficiency. Adequate P is essential for proper bone development and mineralization and thus this data further suggests a P deficiency in pigs fed diets including phytase relative to the treatments given high or NRC levels of STTD P. However, in Exp. 2 there was no differences in 25-OH-D3, as P could still be considered adequate in the later finishing phases when monocalcium P was already completely removed, as previously described. All of the aforementioned concerns could have played a role in the results of these experiments.

Using the ADFI and STTD P level in diet formulation for each treatment, calculations were made for grams of STTD P intake per day. These calculations indicate that assuming pigs had 135 g mean protein deposition (assumption of NRC, 2012), the STTD P level in the diet was sufficient to meet 115 to 125% of the NRC requirement estimate (g/d) for the NRC STTD P treatment. Pigs fed diets with high levels of STTD P had 137 to 158% of the NRC requirement estimate (g/d). Comparing the intakes in the current study to that of Vier et al. (2019), pigs in our study had increased ADFI, thus pigs had additional STTD P intake compared to pigs in Vier et al. (2019). Furthermore, the increased growth and bone mineralization seen in Vier et al. (2019) up to 130% of NRC requirement estimates could be a result of lower ADFI.

In the current study, there were no further improvements in growth performance or bone characteristics when pigs were fed high levels of STTD P vs NRC (2012) requirement estimates. Vier et al. (2019) observed improvements in both growth and bone mineralization when pigs were fed increasing STTD P up to 130% of the NRC (2012) requirement estimate. Many other studies corroborate these findings that the STTD P requirement is somewhat higher than that of the NRC (2012) estimate based on improvements in growth performance when feeding STTD P to a higher level than the current NRC (2012) requirement estimate (Lagos et al., 2019; Lautrou et al., 2020). As previously mentioned, the pigs fed the NRC levels of STTP diet had a g/d intake of STTD P that was likely already greater than NRC (2012) estimates, and thus perhaps pigs fed increased STTD P on the high STTD P treatment saw no further performance or bone mineralization improvements as a result.

In conclusion, regardless of diet formulation strategy there was decreased performance or bone mineralization observed for pigs fed diet containing phytase relative to pigs fed diets with only an inorganic P source. In Exp. 1, there was a significant decrease in ADG, G:F, and 25-OH-D3 in pigs fed phytase compared to pigs fed NRC STTD P. In Exp. 2, while no statistical differences were observed between treatments for overall growth performance, pigs fed diets with phytase had decreased metacarpal bone density and percentage bone ash relative to pigs fed diets with NRC or high STTD P. These findings ultimately suggest that the full nutrient release values attributed to the phytase were overestimated and, consequently, resulted in poorer performance and bone mineralization.

References

AOAC. 2023. Official methods of analysis AOAC international. 22nd ed. Association of Official Analytical Chemists, Arlington, VA.

Augspurger, N. R., D. M. Webel, and D. H. Baker. 2007. An Escherichia coli phytase expressed in yeast effectively replaces inorganic phosphorus for finishing pigs and laying hens. J. Anim. Sci. 85(5):1192-1198. doi:10.2527/jas.2006-340.

Becker, S. L., S. A. Gould, A. L., Petry, L. M. Kellesvig, and J. F. Patience. 2020. Adverse effects on growth performance and bone development in nursery pigs fed diets marginally deficient in phosphorus with increasing calcium to available phosphorus ratios. J. Anim. Sci. 98:1–8. doi:10.1093/jas/skaa325.

Cromwell, G. L., T. S. Stahly, R. D. Coffey, H. J. Monegue, and J. H. Randolph. 1993. Efficacy of phytase in improving bioavailability of phosphorus in soybean meal and corn-soybean meal diets for pigs. J. Anim. Sci. 71:1831-1840. doi:10.2527/1993.7171831.

Espinosa, C. D., L. J. Torres, D. E. Velayudhan, Y. Dersjant-Li, and H. H. Stein. 2022. Ileal and total tract digestibility of energy and nutrients in pig diets supplemented with a novel consensus bacterial 6-phytase variant. J. Anim. Sci. 100:skac364. doi:10.1093/jas/skac364.

Gonçalves, M. A. D,, S. S. Dritz, M. D. Tokach, DeRouchey, J. M., Woodworth, J. C., and R. D. Goodband. 2016. Fact sheets–comparing phytase sources for pigs and effects of superdosing phytase on growth performance of nursery and finishing pigs. J. Swine Health Prod. 24(2):97-101.

Gonzalez-Vega, J. C., Y. Liu, J. C. McCann, C. L. Walk, J. J. Loor, and H. H. Stein. 2016. Requirement for digestible calcium by eleven- to twenty-five-kilogram pigs as determined by growth performance, bone ash concentration, calcium and phosphorus balances, and expression of genes involved in transport of calcium in intestinal and kidney cells. J. Anim. Sci. 94:3321-3334. doi:10.2527/jas.2016-0444.

Igbasan, F. A., K. Männer, G. Miksch, R., Borriss, A. Farouk, and O. Simon. 2000. Comparative studies on the in vitro properties of phytases from various microbial origins. Archives of Animal Nutrition, 53(4):353-373. doi:10.1080/17450390009381958.

Jones, G. 2007. Expanding role for vitamin D in chronic kidney disease: importance of blood 25-OH-D levels and extra-renal 1a-hydroxylase in the classical and nonclassical actions of 1a, 25-dihydroxyvitamin D3. In Seminars in dialysis. 20(4):316. Blackwell Publishing Ltd. doi:10.1111/j.1525-139X.2007.00302.x.

Lagos, L. V., C. L. Walk, M. R. Murphy, and H. H. Stein. 2019. Effects of dietary digestible calcium on growth performance and bone ash concentration in 50-to 85-kg growing pigs fed diets with different concentrations of digestible phosphorus. Anim. Feed Sci. Technol. 247:262-272. doi:10.1016/j.anifeedsci.2018.11.019

Lagos, L. V., M. R. Bedford and H. H. Stein. 2022. Apparent digestibility of energy and nutrients and efficiency of microbial phytase is influenced by body weight of pigs. J. Anim. Sci. 100:skac269. doi:10.1093/jas/skac269.

Lee, S. A., and H. H. Stein. 2021. Analyzed values for P and phytate in feed ingredients. Stein Nutrition Newsletter. June, 2021. https://nutrition.ansci.illinois.edu/node/1753 (accessed April, 2023)

Lei, X. G., P. K. Ku, E. R. Miller, M. T. Yokoyama, and D. E. Ullrey. 1994. Calcium level affects the efficacy of supplemental microbial phytase in corn–soybean meal diets of weanling pigs. J. Anim. Sci. 72:139–143. doi:10.2527/1994.721139x.

Liu, J., D. W. Bollinger, D. R. Ledoux, and T. L. Veum. 1998. Lowering the dietary calcium to total phosphorus ratio increases phosphorus utilization in low-phosphorus corn-soybean meal diets supplemented with microbial phytase for growing-finishing pigs. J. Anim. Sci. 76:808–813. doi:10.2527/1998.763808x.

Mullaney, E. J., C. B. Daly, and A. H. J. Ullah. 2000. Advances in phytase research. Adv. Appl. Microbiol. 47:157–199. doi:10.1016/s0065-2164(00)47004-8.

O'Doherty, J. V., D. A. Gahan, C. O'Shea, J. J. Callan, and K. M. Pierce. 2010. Effects of phytase and 25-hydroxyvitamin D_3 inclusions on the performance, mineral balance and bone parameters of grower-finisher pigs fed low-phosphorus diets. Animal. 4:1634–1640. doi:10.1017/S1751731110000807

Olsen, K. M., S. A. Gould, and J. F. Patience. 2021. A comparison of the release of phosphorus by a phytase enzyme in pigs fed diets deficient or adequate in phosphorus content. J. Anim. Sci. 99:skab001. doi:10.1093/jas/skab001.

Qian, H., E. T. Kornegay, and D. E. Conner Jr. 1996. Adverse effects of wide calcium: phosphorus ratios on supplemental phytase efficacy for weanling pigs fed two dietary phosphorus levels. J. Anim. Sci. 74(6):1288-1297. doi:10.2527/1996.7461288x.

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:10.1016/0308-8136(94)90109-0.

Rutherford, S. M., T. K. Chung, and P. J. Moughan. 2014. Effect of microbial phytase on phytate P degradation and apparent digestibility of total P and Ca throughout the gastrointestinal tract of the growing pig. J. Anim. Sci. 92:189-197. doi:10.2527/jas2013-6923.

Selle, P. H., and V. Ravindran. 2008. Phytate-degrading enzymes in pig nutrition. Livest. Sci. 113:99-122. doi:10.1016/j.livsci.2009.01.006.

Selle, P. H., A. J. Coweison, and V. Ravindran. 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livest. Sci. 124:126-141. doi:10.1016/j.livsci.2009.01.006.

Thorsen, M., Nielsen, L. A., Zhai, H. X., Zhang, Q., Wulf-Andersen, L., and L. K. Skov. 2021. Safety and efficacy profile of a phytase produced by fermentation and used as a feed additive. Heliyon. 7(6):e07237. doi:10.1016/j.heliyon.2021.e07237.

Vier, C. M., S. S. Dritz., F. Wu, M.D Tokach, J.M. DeRouchey, R. D. Goodband, 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.

Wensley, M. R., C. M. Vier, J. T. Gebhardt, M. D. Tokach, J. C. Woodworth, R. D. Goodband, and J. M. DeRouchey. 2020a. Technical Note: Assessment of two methods for estimating bone ash in pigs. J. Anim. Sci. 98:1-8. doi:10.1093/jas/skaa251.

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. 2020b. Determining the phosphorus release of Smizyme TS G5 2,500 phytase in diets for nursery pigs. Transl. Anim. Sci. 4(3):txaa058. doi:10.1093/tas/txaa058.

Williams, H., T. Chin, J. T. Gebhardt, M. D. Tokach, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, J. R. Bergstrom, M. Rahe, C. Siepker, and P. Sitthicharoenchai. 2022. Abstract: The effect of different bone and analytical method on assessment of bone mineralization response to dietary P, phytase, and vitamin D in nursery pigs. J. Anim. Sci. 100:25-25. doi:10.1093/jas/skac064.040.

Zeng, Z. K., X. S. Piao, D. Wang, P. F. Li, L. F. Xue, L. Salmon, and L. Liu. 2011. Effect of microbial phytase on performance, nutrient absorption and excretion in weaned pigs and apparent ileal nutrient digestibility in growing pigs. Asian-Australas. J. Anim. Sci. 24(8):1164-1172. doi:10.5713/ajas.2011.11016.

Zhai, H., J. R. Bergstrom, J. Zhang, W. Dong, Z. Wang, K. Stamatopoulos, and A. J. Cowieson. 2022. Use of fixed calcium to phosphorus ratios in experimental diets may create bias in phytase efficacy responses in swine. Transl. Anim. Sci. 6(3):txax124. doi:10.1093/tas/txac124.

Zhai, H., J. Zhang, Z. Wang, S. Wang, S. Prasad, K. Stamatopoulos, and S. Duval. 2023a. Comparison of digestible and available phosphorus release values for a novel phytase determined with fecal phosphorus digestibility and bone mineralization in weaner pigs. Anim. Feed Sci. Technol. 115580. doi:10.1016/j.anifeedsci.2023.115580.

Zhai, H., J. Bergstrom, J. Zhang, W. Dong, Z. Wang, K. Stamatopoulos, and A. J. CowiesoN. 2023b. The effects of increasing dietary total Ca/total P ratios on growth performance, Ca and P balance, and bone mineralization in nursery pigs fed diets supplemented with phytase. Transl. Anim. Sci. 7(1):txad006. doi:10.1093/tas/txad006.

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

Item	NRC STTD P	600 FYT/kg Phytase[2]	1,000 FYT/kg Phytase[3]	High STTD P
Ingredient, %				
Corn	73.30	73.95	74.10	72.65
Soybean meal	23.35	23.31	23.30	23.40
Calcium carbonate	0.93	0.95	0.98	1.11
Monocalcium phosphate	0.85	0.20	--	1.25
Sodium chloride	0.40	0.40	0.40	0.40
Liquid lysine, 55%	0.63	0.63	0.63	0.63
DL-Met	0.13	0.12	0.12	0.13
L-Trp	0.03	0.03	0.03	0.03
L-Val	0.09	0.09	0.09	0.09
Thr[4]	0.20	0.20	0.20	0.20
Phytase[5]	--	0.03	0.04	--
Vitamin-trace mineral premix[6]	0.10	0.10	0.10	0.10
Total	100	100	100	100
Calculated analysis				
Standard ileal digestible (SID) amino acids, %				
Lys	1.10	1.10	1.10	1.10
Ile:Lys	56	56	56	56
Leu:Lys	123	124	124	123
Met:Lys	34	34	34	34
Met and Cys:Lys	56	56	56	56
Thr:Lys	62	62	62	62
Trp:Lys	18.6	18.6	18.6	18.6
Val:Lys	70	70	70	70
His:Lys	38	38	38	38
Total Lys, %	1.22	1.22	1.22	1.22
NE, kcal/kg	2,421	2,436	2,441	2,405
SID Lys:NE, g/Mcal	4.44	4.41	4.40	4.47
STTD Ca, %[7]	0.40	0.41	0.40	0.50
STTD P, %	0.31	0.31	0.31	0.38
STTD Ca:STTD P	1.30	1.30	1.30	1.30
Ca, %	0.58	0.47	0.45	0.72
P, %	0.54	0.40	0.36	0.63
Ca:P	1.07	1.17	1.24	1.15
Chemical analysis, %				
DM	85.18	84.78	85.06	85.17
CP	17.10	16.10	15.70	14.70

Ca	0.89	0.64	0.67	0.69
P	0.47	0.35	0.26	0.64

[1]Phase 1 was fed from approximately 36 to 50 kg. Diets were analyzed for phytase concentrations (DSM Laboratory, Belvidere, NJ) – 600 and 1,000 FYT/kg actual phytase units were 895 and 1,183 FYT/kg respectively.

[2]Considering release of 0.13% STTD P and 0.095% STTD Ca.

[3]Considering release of 0.16% STTD P and 0.107% STTD Ca.

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

[5]HiPhorius 10 2,400 (DSM, Parsippany, NJ).

[6]Provided per kg of diet: 5,292 IU vitamin A; 1,323 IU vitamin D; 26.5 IU vitamin E; 2.7 mg vitamin K; 0.02 mg vitamin B12; 49.6 mg niacin; 16.5 mg pantothenic acid; 5 mg riboflavin; 16.5 mg Cu; 0.3 mg iodine; 110.0 mg iron; 33.1 mg manganese; 0.3 mg selenium; 110.0 mg zinc.

[7]Standardized total tract digestible.

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

Item	NRC STTD P	600 FYT/kg Phytase[2]	1,000 FYT/kg Phytase[3]	High STTD P
Ingredient, %				
Corn	79.71	80.44	80.37	79.23
Soybean meal	17.29	17.23	17.24	17.32
Calcium carbonate	0.86	0.85	0.95	1.00
Monoalcium phosphate	0.75	0.05	--	1.05
Sodium chloride	0.40	0.40	0.40	0.40
Liquid lysine, 55%	0.57	0.57	0.57	0.57
DL-Met	0.07	0.07	0.07	0.07
L-Trp	0.03	0.03	0.03	0.03
L-Val	0.07	0.07	0.07	0.07
Thr[4]	0.16	0.16	0.16	0.16
Phytase[5]	--	0.03	0.04	--
Vitamin-trace mineral premix[6]	0.10	0.10	0.10	0.10
Total	100	100	100	100
Calculated analysis				
Standard ileal digestible (SID) amino acids, %				
Lys	0.92	0.92	0.92	0.92
Ile:Lys	56	56	56	56
Leu:Lys	132	133	133	132
Met:Lys	32	32	32	32
Met and Cys:Lys	56	56	56	56
Thr:Lys	62	62	62	62
Trp:Lys	18.6	18.6	18.6	18.6
Val:Lys	70	70	70	70
His:Lys	39	39	39	39
Total Lys, %	1.03	1.03	1.03	1.03
NE, kcal/kg	2,465	2,485	2,482	2,454
SID Lys:NE, g/Mcal	3.66	3.63	3.63	3.67
STTD Ca, %[7]	0.35	0.35	0.38	0.43
STTD P, %	0.27	0.27	0.29	0.33
STTD Ca:STTD P	1.30	1.30	1.30	1.30
Ca, %	0.52	0.39	0.42	0.62
P, %	0.49	0.34	0.33	0.56
Ca:P	1.05	1.14	1.25	1.12
Chemical analysis, %				
DM	85.60	85.51	85.58	85.67
CP	12.10	14.70	14.0	13.6

Ca	0.57	0.50	0.53	0.56
P	0.41	0.31	0.27	0.51

[1]Phase 2 was fed from approximately 50 to 75 kg.

[2]Considering release of 0.13% STTD P and 0.095% STTD Ca.

[3]Considering release of 0.16% STTD P and 0.107% STTD Ca.

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

[5]HiPhorius 10 2,400 (DSM, Parsippany, NJ).

[6]Provided per kg of diet: 5,292 IU vitamin A; 1,323 IU vitamin D; 26.5 IU vitamin E; 2.7 mg vitamin K; 0.02 mg vitamin B12; 49.6 mg niacin; 16.5 mg pantothenic acid; 5 mg riboflavin; 16.5 mg Cu; 0.3 mg iodine; 110.0 mg iron; 33.1 mg manganese; 0.3 mg selenium; 110.0 mg zinc.

[7] Standardized total tract digestible.

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

Item	NRC STTD P	600 FYT/kg Phytase[2]	1,000 FYT/kg Phytase[3]	High STTD P
Ingredient, %				
Corn	83.76	84.41	84.28	83.37
Soybean meal	13.58	13.53	13.54	13.60
Calcium carbonate	0.80	0.82	0.93	0.91
Monocalcium phosphate	0.65	--	--	0.90
Sodium chloride	0.40	0.40	0.40	0.40
Liquid lysine, 55%	0.50	0.50	0.50	0.50
DL-Met	0.03	0.03	0.03	0.03
L-Trp	0.03	0.03	0.03	0.03
L-Val	0.03	0.03	0.03	0.03
Thr[4]	0.13	0.13	0.13	0.13
Phytase[5]	--	0.03	0.04	--
Vitamin-trace mineral premix[6]	0.10	0.10	0.10	0.10
Total	100	100	100	100
Calculated analysis				
Standard ileal digestible (SID) amino acids, %				
Lys	0.79	0.79	0.79	0.79
Ile:Lys	57	57	57	57
Leu:Lys	143	144	144	143
Met:Lys	30	30	30	30
Met and Cys:Lys	56	56	56	56
Thr:Lys	63	63	63	63
Trp:Lys	18.5	18.5	18.5	18.5
Val:Lys	70	70	70	70
His:Lys	41	41	41	41
Total Lys, %	0.89	0.89	0.89	0.89
NE, kcal/kg	2,496	2,511	2,509	2,485
SID Lys:NE, g/Mcal	3.11	3.09	3.09	3.12
STTD Ca, %[7]	0.32	0.33	0.37	0.38
STTD P, %	0.24	0.25	0.28	0.29
STTD Ca:STTD P	1.30	1.30	1.30	1.30
Ca, %	0.47	0.36	0.40	0.55
P, %	0.46	0.32	0.32	0.51
Ca:P	1.03	1.13	1.26	1.09
Chemical analysis, %				
DM	84.93	84.74	85.92	84.89
CP	11.10	12.40	12.90	11.20

Ca	0.55	0.33	0.34	0.56
P	0.42	0.25	0.27	0.49

[1]Phase 3 was fed from approximately 75 to 100 kg. Diets were analyzed for phytase concentrations (DSM Laboratory, Belvidere, NJ) – 600 and 1000 FYT/kg actual phytase units were 845 and 1,286 FYT/kg respectively.

[2]Considering release of 0.13% STTD P and 0.095% STTD Ca.

[3]Considering release of 0.16% STTD P and 0.107% STTD Ca.

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

[5]HiPhorius 10 2,400 (DSM, Parsippany, NJ).

[6]Provided per kg of diet: 5,292 IU vitamin A; 1,323 IU vitamin D; 26.5 IU vitamin E; 2.7 mg vitamin K; 0.02 mg vitamin B12; 49.6 mg niacin; 16.5 mg pantothenic acid; 5 mg riboflavin; 16.5 mg Cu; 0.3 mg iodine; 110.0 mg iron; 33.1 mg manganese; 0.3 mg selenium; 110.0 mg zinc.

[7]Standardized total tract digestible.

Table 2.4. Diet composition (as-fed basis) Phase 4, Exp. 1[1]

Item	NRC STTD P	600 FYT/kg Phytase[2]	1,000 FYT/kg Phytase[3]	High STTD P
Ingredient, %				
Corn	86.86	87.27	87.13	86.46
Soybean meal	10.70	10.67	10.68	10.72
Calcium carbonate	0.72	0.81	0.93	0.84
Monocalcium phosphate	0.59	--	--	0.75
Sodium chloride	0.40	0.40	0.40	0.40
Liquid lysine, 55%	0.50	0.50	0.50	0.50
DL-Met	0.04	0.04	0.04	0.04
L-Trp	0.03	0.03	0.03	0.03
L-Val	0.03	0.03	0.03	0.03
Thr[4]	0.14	0.14	0.14	0.14
Phytase[5]	--	0.03	0.04	--
Vitamin-trace mineral premix[6]	0.10	0.10	0.10	0.10
Total, %	100	100	100	100
Calculated analysis				
Standard ileal digestible (SID) amino acids, %				
Lys	0.72	0.72	0.72	0.72
Ile:Lys	56	56	56	56
Leu:Lys	148	148	148	148
Met:Lys	32	32	32	32
Met and Cys:Lys	59	59	59	59
Thr:Lys	65	65	65	65
Trp:Lys	18.8	18.8	18.8	18.8
Val:Lys	70	70	70	70
His:Lys	41	41	41	41
Total Lys, %	0.81	0.81	0.81	0.81
NE, kcal/kg	2,520	2,531	2,527	2,509
SID Lys:NE, g/Mcal	2.81	2.80	2.80	2.82
CP	12.80	12.80	12.80	12.70
STTD Ca, %[7]	0.27	0.32	0.36	0.33
STTD P, %	0.21	0.24	0.27	0.26
STTD Ca:STTD P	1.30	1.30	1.30	1.30
Ca, %	0.40	0.35	0.39	0.49
P, %	0.41	0.30	0.30	0.46
Ca:P	0.98	1.14	1.28	1.06

[1]Phase 4 was fed from approximately 100 to 136 kg.
[2]Considering release of 0.13% STTD P and 0.095% STTD Ca.
[3]Considering release of 0.16% STTD P and 0.107% STTD Ca.

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

[6]Provided per kg of diet: 5,292 IU vitamin A; 1,323 IU vitamin D; 26.5 IU vitamin E; 2.7 mg vitamin K; 0.02 mg vitamin B12; 49.6 mg niacin; 16.5 mg pantothenic acid; 5 mg riboflavin; 16.5 mg Cu; 0.3 mg iodine; 110.0 mg iron; 33.1 mg manganese; 0.3 mg selenium; 110.0 mg zinc.

[7] Standardized total tract digestible.

Table 2.5. Diet composition (as-fed basis) Phase 1, Exp. 2[1]

Item	NRC STTD P	600 FYT/kg Phytase[2]	1,000 FYT/kg Phytase[3]	High STTD P
Ingredient, %				
Corn	83.60	84.40	84.38	83.28
Soybean meal	13.59	13.53	13.53	13.61
Calcium carbonate	0.95	0.83	0.83	1.00
Monocalcium phosphate	0.65	--	--	0.90
Sodium chloride	0.40	0.40	0.40	0.40
Liquid lysine, 55%	0.50	0.50	0.50	0.50
DL-Met	0.03	0.03	0.03	0.03
L-Trp	0.03	0.03	0.03	0.03
L-Val	0.03	0.03	0.03	0.03
Thr[4]	0.13	0.13	0.13	0.13
Phytase[5]	--	0.03	0.04	--
Vitamin-trace mineral premix[6]	0.10	0.10	0.10	0.10
Total, %	100	100	100	100
Calculated analysis				
Standard ileal digestible (SID) amino acids, %				
Lys	0.79	0.79	0.79	0.79
Ile:Lys	57	57	57	57
Leu:Lys	143	144	144	143
Met:Lys	30	30	30	30
Met and Cys:Lys	56	56	56	56
Thr:Lys	63	63	63	63
Trp:Lys	18.5	18.5	18.5	18.5
Val:Lys	70	70	70	70
His:Lys	41	41	41	41
Total Lys, %	0.89	0.89	0.89	0.89
NE, kcal/kg	2,538	2,557	2,557	2,529
SID Lys:NE, g/Mcal	3.11	3.09	3.09	3.12
CP	13.90	13.90	13.90	13.80
STTD P[7], %	0.24	0.25	0.28	0.29
STTD Ca:STTD P	1.44	1.31	1.22	1.37
Ca, %	0.52	0.36	0.36	0.59
P, %	0.45	0.32	0.32	0.51
Ca:P	1.15	1.15	1.15	1.15

[1]Phase 1 was fed from approximately 75 to 100 kg.
[2]Considering release of 0.13% STTD P and 0.095% STTD Ca.
[3]Considering release of 0.16% STTD P and 0.107% STTD Ca.
[4]Thr Pro; CJ America-Bio, Downers Grove, IL.

[5]HiPhorius 10 2400 (DSM, Parsippany, NJ).

[6]Provided per kg of diet: 5,292 IU vitamin A; 1,323 IU vitamin D; 26.5 IU vitamin E; 2.7 mg vitamin K; 0.02 mg vitamin B12; 49.6 mg niacin; 16.5 mg pantothenic acid; 5 mg riboflavin; 16.5 mg Cu; 0.3 mg iodine; 110.0 mg iron; 33.1 mg manganese; 0.3 mg selenium; 110.0 mg zinc.

[7] Standardized total tract digestible.

Table 2.6. Diet composition (as-fed basis) Phase 2, Exp. 2[1]

Item	NRC STTD P	600 FYT/kg Phytase[2]	1,000 FYT/kg Phytase[3]	High STTD P
Ingredient, %				
Corn	86.69	87.29	87.27	86.37
Soybean meal	10.71	10.67	10.67	10.73
Calcium carbonate	0.88	0.79	0.79	0.92
Monocalcium phosphate	0.50	--	--	0.75
Sodium chloride	0.40	0.40	0.40	0.40
Liquid lysine, 55%	0.50	0.50	0.50	0.50
DL-Met	0.04	0.04	0.04	0.04
L-Trp	0.03	0.03	0.03	0.03
L-Val	0.03	0.03	0.03	0.03
Thr[4]	0.14	0.14	0.14	0.14
Phytase[5]	--	0.03	0.04	--
Vitamin-trace mineral premix[6]	0.10	0.10	0.10	0.10
Total, %	100	100	100	100
Calculated analysis				
Standard ileal digestible (SID) amino acids, %				
Lys	0.72	0.72	0.72	0.72
Ile:Lys	56	56	56	56
Leu:Lys	148	148	148	148
Met:Lys	32	32	32	32
Met and Cys:Lys	59	59	59	59
Thr:Lys	65	65	65	65
Trp:Lys	18.8	18.8	18.8	18.8
Val:Lys	70	70	70	70
His:Lys	41	41	41	41
Total Lys, %	0.81	0.81	0.81	0.81
NE, kcal/kg	2,560	2,575	2,575	2,551
SID Lys:NE, g/Mcal	2.81	2.80	2.80	2.82
STTD P[7], %	0.21	0.24	0.27	0.26
STTD Ca:STTD P	1.47	1.28	1.19	1.37
Ca, %	0.46	0.34	0.34	0.52
P, %	0.41	0.30	0.30	0.46
Ca:P	1.12	1.12	1.12	1.12
Chemical analysis, %				
DM	84.90	84.64	84.88	84.90
CP	12.30	11.50	10.80	11.00
Ca	0.57	0.37	0.36	0.74

P	0.45	0.26	0.26	0.52

[1]Phase 2 was fed from approximately 100 to 136 kg.

[2]Considering release of 0.13% STTD P and 0.095% STTD Ca.

[3]Considering release of 0.16% STTD P and 0.107% STTD Ca.

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

[5]HiPhorius 10 2400 (DSM, Parsippany, NJ).

[6]Provided per kg of diet: 5,292 IU vitamin A; 1,323 IU vitamin D; 26.5 IU vitamin E; 2.7 mg vitamin K; 0.02 mg vitamin B12; 49.6 mg niacin; 16.5 mg pantothenic acid; 5 mg riboflavin; 16.5 mg Cu; 0.3 mg iodine; 110.0 mg iron; 33.1 mg manganese; 0.3 mg selenium; 110.0 mg zinc.

[7] Standardized total tract digestible.

Table 2.7. Effects of added HiPhorius phytase on finishing pig growth performance and carcass characteristics, Exp. 1[1]

	NRC STTD P[2]	600 FYT/kg Phytase	1,000 FYT/kg Phytase	High STTD P	SEM	*P* =
BW, kg						
d 0	36.7	36.7	36.7	36.7	0.48	0.999
d 54	99.5^{a}	95.8^{b}	97.6^{ab}	100.3^{a}	0.87	0.003
d 105	136.3^{a}	134.7^{b}	132.5^{b}	135.7^{ab}	0.97	0.005
Grower phase, d 0 to 54						
ADG, kg	0.89^{xy}	0.86^{y}	0.86^{y}	0.91^{x}	0.031	0.030
ADFI, kg	2.31	2.29	2.34	2.39	0.031	0.184
G:F	0.385^{x}	0.375^{xy}	0.369^{y}	0.381^{xy}	0.004	0.071
Finisher phase, d 54 to 105						
ADG, kg	1.04	0.99	1.00	1.01	0.019	0.250
ADFI, kg	3.32	3.27	3.31	3.33	0.040	0.658
G:F	0.313	0.301	0.303	0.303	0.005	0.264
Overall (d 0 to 105)						
ADG, kg	0.96^{a}	0.92^{b}	0.93^{b}	0.96^{a}	0.007	< 0.001
ADFI, kg	2.79	2.75	2.79	2.83	0.026	0.159
G:F	0.345^{b}	0.334^{ab}	0.332^{a}	0.338^{ab}	0.003	0.025
Carcass characteristics[3]						
HCW, kg[4]	100.3	98.1	98.2	100.1	0.730	0.047
Carcass yield, %	74.0	74.0	73.7	73.8	0.003	0.775
Loin depth, cm	6.50	6.38	6.35	6.43	0.045	0.177
Back fat depth, cm	1.65^{x}	1.73^{y}	1.73^{y}	1.72^{xy}	0.023	0.061
Lean, %	56.62^{a}	55.98^{b}	55.96^{b}	56.14^{ab}	0.162	0.021

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

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

[1] A total of 1,161 pigs (initially 36.7 ± 0.481 kg) were used in in a 105-d finisher trial with 27 pigs per pen and 10 or 11 pens per treatment. Pigs were allotted to treatment in a completely randomized design. Treatments consisted of: 1) No phytase and formulated to NRC requirement estimates for STTD P; 2) 600 FYT/kg added HiPhorius phytase formulated to the same STTD Ca and P as treatment 1 considering a release of 0.13% STTD P and 0.095% STTD Ca; 3) 1,000 FYT/kg HiPhorius phytase formulated to the same STTD Ca and P as treatment 1 considering release of 0.16% STTD P and 0.107% STTD Ca, and 4) high levels for STTD P (no phytase; approximately 22% above NRC). All diets were formulated to the same STTD Ca: STTD P ratio.

[2] Standardized total tract digestible.

[3]In the analysis for backfat depth, lean %, and loin depth, HCW was used as a covariate in the model.

[4]When performing pairwise comparisons using a Tukey multiple comparison adjustment, no comparisons were ($P < 0.05$). However, the greatest difference between dietary treatments was between pigs fed diets formulated with 600 FYT/kg of phytase compared to pigs fed NRC levels of STTD P, which did not significantly differ ($P = 0.131$).

Table 2.8. Effects of added HiPhorius phytase on finishing pig serum chemistry, Exp. 1[1]

Item	NRC STTD P[2]	600 FYT/kg Phytase	1,000 FYT/kg Phytase	High STTD P	SEM	*P* =
Mineral						
Ca, ppm	108.2	110.0	109.4	107.1	1.79	0.671
Cu, ppm	2.3	2.1	2.1	2.2	0.10	0.504
Fe, ppm	1.9	1.9	2.3	1.9	0.26	0.482
K, ppm	195.3	191.2	183.1	193.9	7.59	0.644
Mg, ppm	19.6	19.6	19.7	19.3	0.54	0.950
Mn, ppb	2.6	2.6	2.6	2.7	0.22	0.966
Mo, ppb	11.5	12.0	12.0	11.4	0.99	0.947
P, ppm	52.5	50.1	52.5	51.6	1.13	0.354
Se, ppb	252.2	241.5	238.8	243.2	6.62	0.480
Zn, ppm	1.1	1.1	1.1	1.1	0.05	0.962
25-Hydroxyvitamin-D3, ng/ml	34.7[x]	28.2[y]	28.2[y]	32.9[xy]	1.82	0.022
1,25-Dihydroxyvitamin-D3, pg/ml	203.8	188.4	198.2	153.0	20.65	0.303

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

[1] A total of 1,161 pigs (initially 36.7 ± 0.481 kg) were used in in a 105-d finisher trial with 27 pigs per pen and 10 or 11 pens per treatment. Pigs were allotted to treatment in a completely randomized design. Treatments consisted of: 1) No phytase and formulated to NRC requirement estimates for STTD P; 2) 600 FYT/kg added HiPhorius phytase formulated to the same STTD Ca and P as treatment 1 considering a release of 0.13% STTD P and 0.095% STTD Ca; 3) 1,000 FYT/kg HiPhorius phytase formulated to the same STTD Ca and P as treatment 1 considering release of 0.16% STTD P and 0.107% STTD Ca, and 4) high levels for STTD P (no phytase; approximately 22% above NRC). All diets were formulated to the same STTD Ca: STTD P ratio. Serum samples were collected from one pig per pen approximately 12 h prior to harvest and analyzed at the ISU-VDL and Heartland assays (Ames, IA).

[2]Standardized total tract digestible.

Table 2.9. Effects of feeding HiPhorius phytase on finisher growth performance and carcass characteristics, Exp. 2[1]

	NRC STTD P[2]	600 FYT/kg Phytase	1,000 FYT/kg Phytase	High STTD P	SEM	*P* =
BW, kg						
d 0	76.0	76.0	76.0	76.0	1.32	0.999
d 17	93.7	92.0	93.7	93.4	1.34	0.788
d 45	121.8	119.8	122.3	121.8	1.16	0.453
d 55	132.3	130.0	133.3	132.6	1.20	0.255
Period 1, d 0 to 17						
ADG, kg	1.06[a]	0.95[b]	1.04[ab]	1.03[ab]	0.024	0.015
ADFI, kg	2.80[a]	2.59[b]	2.74[ab]	2.75[ab]	0.043	0.009
G:F	0.378	0.366	0.378	0.374	0.008	0.615
Period 2, d 17 to 45						
ADG, kg	1.00	0.99	1.01	1.01	0.021	0.853
ADFI, kg	3.23	3.20	3.28	3.20	0.045	0.535
G:F	0.309	0.309	0.309	0.315	0.004	0.626
Period 3, d 45 to 55						
ADG, kg	0.99	0.96	1.02	1.00	0.025	0.420
ADFI, kg	3.52	3.45	3.48	3.64	0.061	0.163
G:F	0.282	0.279	0.293	0.276	0.007	0.369
Overall, d 0 to 55						
ADG, kg	1.02	0.97	1.02	1.01	0.017	0.175
ADFI, kg	3.16	3.07	3.16	3.15	0.036	0.216
G:F	0.321	0.317	0.323	0.322	0.004	0.673
Carcass characteristics[3]						
HCW, kg	98.7	98.0	99.2	99.2	0.840	0.701
Carcass yield, %	74.0	74.6	74.0	74.3	0.004	0.684
Loin depth, cm	6.43	6.45	6.50	6.45	0.021	0.807
Back fat depth, cm	1.83	1.80	1.80	1.83	0.013	0.705
Lean, %	55.47	55.70	55.77	55.41	0.225	0.622

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

[1] A total of 1,160 pigs (initially 76.0 ± 1.32 kg**)** were used in in a 105-d finisher trial with 27 pigs per pen and 11 pens per treatment. Pigs were allotted to treatment in a completely randomized design Treatments consisted of: (1) No phytase, formulated to NRC requirement for Ca and STTD P, (2) Diet + 600 FYT/kg formulated to the same Ca and STTD P as treatment 1 considering release of 0.13% STTD P (3) Diet + 1,000 FYT/kg formulated to the same Ca and STTD P as treatment 1 considering release of 0.16% STTD P 4) industry levels for STTD P (no phytase; approximately 22% above NRC). All diets were formulated to the same analyzed Ca:P ratio.

[2]Standardized total tract digestible.

[3]In the analysis for backfat depth, lean, and loin depth, HCW was used as a covariate in the model.

Table 2.10. Effects of feeding HiPhorius phytase on finishing pig metacarpal bone mineralization and serum chemistry, Exp. 2[1]

Item	NRC STTD P[2]	600 FYT/kg Phytase	1,000 FYT/kg Phytase	High STTD P	SEM	*P* =
Bone density, g/mL	1.35[a]	1.31[b]	1.33[ab]	1.36[a]	0.008	0.001
Bone ash weight, g	9.27	8.23	8.98	9.73	0.394	0.057
Bone ash, %	58.5	54.4	56.7	59.0	0.013	0.060
Ca, g	3.91	3.82	4.08	4.29	0.276	0.587
Ca, %	42.1	46.0	45.4	43.9	1.810	0.447
P, g	1.61	1.56	1.67	1.74	0.115	0.715
P, %	17.3	18.8	18.6	17.8	0.900	0.572
25-Hydroxyvitamin-D3, ng/ml	28.73	29.00	27.29	30.45	2.014	0.745

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

[1]A total of 1,160 pigs (initially 76.0 ± 1.32 kg**)** were used in in a 105-d finisher trial with 27 pigs per pen and 11 pens per treatment. Pigs were allotted to treatment in a completely randomized design. Treatments consisted of: (1) No phytase, formulated to NRC requirement for Ca and STTD P, (2) Diet + 600 FYT/kg formulated to the same Ca and STTD P as the PC considering release of 0.13% STTD P, (3) Diet + 1,000 FYT/kg formulated to the same Ca and STTD P as the PC considering release of 0.16% STTD P 4) high levels for STTD P (no phytase; approximately 22% above NRC) by using added monocalcium P and limestone to provide 14% Ca and 21% STTD P above NRC (2012) requirements. All diets were formulated to the same analyzed Ca:P ratio. Serum samples were collected from one pig per pen approximately 12 h prior to harvest and analyzed at the ISU-VDL (Ames, IA). On one pig per pen the right foot was collected, and third metacarpals were used to determine bone density, and fourth metacarpals were used for bone ash and bone Ca and P.

[2]Standardized total tract digestible.

Chapter 3 - Evaluation of HiPhorius phytase on growth performance and bone mineralization of nursery pigs

Abstract

A total of 297 pigs (DNA 241 × 600; initially 8.64 ± 0.181 kg) were used in a 21-d trial. All pigs were fed P depletion diets (0.09% aP) for a 3-d period before the start of the study (d 14 after weaning). Pens of pigs were then assigned to 1 of 5 treatments in a randomized complete block design with 5 pigs per pen and 12 replications per treatment. All pigs were fed aP depletion diets (0.09% aP) for a 3-d period before the start of the study (d 14 after weaning). The first three diets were formulated to contain 0.09% aP; without added phytase (control), or control with 600 or 1,000 FYT/kg of added phytase (considering a release of 0.15 or 0.18% aP, respectively). The remaining two diets were formulated to contain 0.27% aP, one without added phytase and the other with 1,000 FYT/kg. From d 0 to 21, pigs fed diets with increasing phytase containing 0.09% aP had increased (linear $P \leq 0.002$) ADG, ADFI, and G:F. Increasing phytase in diets containing 0.09% aP increased percentage bone ash in metacarpals and 10th ribs (linear, $P < 0.001$; quadratic, $P = 0.004$, respectively), and increased grams of Ca and P in all three bones (linear, $P \leq 0.027$). Each bone showed increased percentage bone ash ($P \leq 0.038$) and increased grams of Ca and P in fibulas and 10th ribs ($P \leq 0.023$) when adding 1,000 FYT/kg phytase in diets with 0.27% aP compared with pigs fed 0.27% aP without added phytase. Increasing aP from 0.09% to 0.27% in diets without added phytase increased ($P < 0.001$) ADG, ADFI, and G:F. Increasing aP from 0.09% to 0.27% in diets without added phytase increased bone density ($P \leq 0.002$) in fibulas and metacarpals, percentage bone ash in all bones ($P \leq 0.074$), and increased ($P < 0.05$) grams of Ca and P in fibulas and 10th ribs. Comparing pigs fed diets containing either 0.09 or 0.27% aP with 1,000 FYT phytase, increasing the aP level also

increased bone density ($P \leq 0.008$) in fibulas and metacarpals, percentage bone ash in all bones ($P \leq 0.002$), and also increased ($P < 0.05$) grams of Ca and P in fibulas and 10th ribs. For growth performance (average of ADG and G:F), aP release was calculated to be 0.170% for 600 FYT/kg and 0.206% for 1,000 FYT/kg. For the average of all 3 bone measurements (average of 3 bones for bone density and percentage bone ash), aP release was calculated to be 0.120% and 0.125% for 600 and 1,000 FYT/kg, respectively.

Key Words: bone ash, bone density, growth, nursery pigs, phosphorus, phytase

List of abbreviations

ADG, average daily gain

ADFI, average daily feed intake

aP, available phosphorus

ATTD, apparent total tract digestibility

BW, body weight

Ca:P, calcium-to-phosphorus ratio

FTU, phytase unit

G:F, gain-to-feed

NE, net energy

SID, standardized ileal digestibility

STTD, standard total tract digestibility

Introduction

Approximately 60 to 80% of phosphorus (P) in feedstuffs of plant origin is stored in the form of phytic acid (Eeckhout et al., 1994). Monogastric species produce little endogenous phytase to cleave the phosphates from the phytate (Cromwell et al., 1993; Selle and Ravindran, 2007). Thus, phytate is commonly considered an antinutritional factor in swine diets as it is associated with reduced P digestibility. Because of the limited availability of P within swine diets from feedstuffs, nutritionists often add inorganic sources of P, such as monocalcium phosphate, to meet the biological requirement for growth and appropriate bone formation. Existing phytase sources can be further developed to improve their efficacy and cost competitiveness. Consequently, new research is required to confirm the assumed P release and suggested improvements in growth and bone mineralization when the next generation phytases become available.

HiPhorius phytase (DSM, Parsippany, NJ) is classified as a 6-phytase and is a modified *C. braakii* phytase expressed from a strain of *A. oryzae*. In recent studies, increasing HiPhorius phytase in P deficient diets improved growth performance and bone mineralization when fed to nursery pigs (Thorsen et al., 2021; Zhai et al., 2023). Their approach to bone mineralization was limited to analyzing one bone (femur or tibia) from each pig and specifically focused on determining bone ash as the primary response criteria to assess bone mineralization. Thus, the objective of the current study was to determine the performance and bone mineralization of nursery pigs fed various levels of HiPhorius phytase in diets with deficient and adequate (NRC, 2012) aP. Diets with adequate aP were formulated to the NRC (2012) requirement estimate for STTD P which equated to 0.27% aP. Based on previous literature, it was hypothesized that when phytase dose is increased in the P deficient diets, an increase in

growth performance and bone mineralization would be observed as additional phytate-bound P is released by the phytase. Literature also shows that increased P is needed to maximize bone characteristics relative to growth performance **(**Cromwell et al., 1970; Augspurger et al., 2003; Wensley et al., 2020b**),** and thus it was further hypothesized that when including phytase in diets with adequate aP, further improvements in bone mineralization would be observed as well as extra-phosphoric effects resulting in an increase in growth performance **(**Dersjant-Li et al., 2015; Arredondo et al. 2019**).**

Materials and Methods

The Kansas State University Animal Care and Use Committee approved the protocol (4485) used in this experiment. The study was conducted at the Kansas State University Research and Teaching Center in Manhattan, KS.

Diet manufacturing

Monocalcium phosphate and calcium carbonate were analyzed in duplicate for Ca and P (Kansas State University Soils Lab). Samples were analyzed by an Inductively Coupled Plasma (ICP) Spectrometer (Model 5800 ICP OES Agilent Technologies, Varian Australia Pty Ltd, Mulgrave, Victoria Australia) to determine nutrient loading values used in diet formulation. Ca and P values for limestone were found to be Ca: 40.11%; P: 0.04%, and Ca: 16.81%; P: 21.16%. for monocalcium phosphate. Sand was used in diets to equalize the batch size and dietary NE among all treatments. The phytase (; DSM Nutritional Products, Parsippany, NJ) was analyzed and contained 11,913 FYT/g (DSM Laboratory, Belvidere, NJ). All diets were formulated to contain a Ca:P ratio of 1.20:1 with no allowance for Ca release by phytase. All diets were corn-soybean meal-based and were manufactured at the O. H. Kruse Feed Technology Innovation

Center at Kansas State University, Manhattan, KS. Complete diet samples were taken during the bagging of experimental diets with a sub-sample collected from every third bag and pooled into one homogenized sample per dietary treatment. After homogenization, samples were stored at -20°C until analysis (KSU Soils Lab in Manhattan, KS) for Ca and P using similar procedures previously described. All diets were formulated to 1.24% SID Lys and to meet or exceed the NRC (2012) requirement estimates for all other nutrients, with the exception of the intended levels of Ca and P (Table 1). Available P values were derived from NRC (1998).

Animals, Housing, and Treatments

A total of 297 pigs (DNA 241 × 600; initially 8.64 ± 0.181 kg) were used in a 21-d trial. Pigs were provided *ab libitum* access to feed and water with each pen containing a 4-hole, dry self-feeder and nipple waterer. At approximately 21 d of age, pigs were weaned and fed common phase 1 and 2 diets for 14 d. All pigs were fed a phosphorus depletion diet (0.09% aP) for a 3-d period before the start of the study and were then assigned to 1 of 5 treatments in a randomized complete block design. The first three treatment diets were formulated to contain 0.09% aP; without added phytase (control), then the control diet with 600 FYT/kg or with 1,000 FYT/kg of added phytase (considering a release of 0.15 or 0.18% aP, respectively). The remaining two treatment diets were formulated to 0.27% aP, one without added phytase and the other with 1,000 FYT/kg phytase added. There were 5 pigs per pen and 12 replications per treatment.

During the experiment, pig and feeders were weighed on d 0 and 21 to determine ADG, ADFI, and G:F. On d 21, one pig from each pen (weighing closest to the mean weight of pigs in the pen) was euthanized. The right fibula, metacarpal, and 10th rib from each pig were collected for determination of bone density, bone ash, and Ca and P. After collection, bones were

individually placed in plastic bags with pen identification and stored at -20°C until analysis. Briefly, bones were cleaned of extraneous soft tissue, and the weight of each bone was recorded and then placed in ultrapure water and vacuumed for 4 h under a negative pressure vacuum at 1.06 kg per cubic centimeter. Bones were then submerged in water and weighed again. Archimedes principle was used to determine bone density; the weight or volume of the liquid was assumed equal to the weight of the initial bone minus the submerged bone weight. Taking the initial bone weight divided by the volume of the liquid we were able to determine bone density on each bone. Next, bones were dried at 105°C for 7 d in a drying oven, weighed, and then ashed in a muffle furnace at 600°C for 24 h to determine total ash weight and percentage ash relative to dried bone weight (Wensley et al., 2020a). Subsequently, Ca and P content within each bone was measured (Williams et al., 2022). Between 0.025 and 0.040 g of bone ash from each bone was added to 100 uL nitric acid. Tubes were placed in a hot block at 16°C for 6 h, diluted 1:10 with ultrapure water and then sent to the KSU Soils Lab where Ca and P quantity was determined.

Using performance and bone analysis data, calculations were made to estimate the aP release values of the phytase for different response criteria. Using the aP levels of the 0.09 and 0.27% aP diets, standard release curves were constructed for each criteria (Wensley et al., 2020b). Marginal intake of aP was then estimated using this curve for both the 600 and 1,000 FYT/kg phytase treatments added to the low aP diet, and then using ADFI, the aP release could be estimated for each response criteria. Release estimates were generated based on growth performance (ADG and G:F) as well as measures of bone mineralization including bone density and percentage bone ash for the fibula, metacarpal, and 10^{th} rib.

Statistical Analysis

Pens of pigs were randomly allotted to treatments using initial pen weight as a blocking factor. Data were analyzed as a randomized complete block design for one-way ANOVA using the lmer function from the lme4 package in R (version 3.5.2 (2-07-2018) with pen as the experimental unit, treatment considered fixed effect, and weight block as a random effect. For all growth performance and bone mineralization analysis, treatments were analyzed to determine the linear and quadratic responses of phytase dose within diets containing 0.09% aP. Contrasts were made to compare diets with no phytase and increasing aP (0.09 % vs 0.27%), diets with 1000 FYT/kg of phytase and increasing aP (0.09 % vs 0.27%), diets with 0.09% aP plus 1,000 FYT/kg phytase versus diets with 0.27% aP, and treatments with 0 versus 1,000 FYT/kg phytase in the diets containing 0.27% aP. Results were considered significant with P-values ≤ 0.05 and were considered marginally significant with P-values $0.05 < P \leq 0.10$.

Results

For the experimental period (d 0 to 21), a linear increase ($P \leq 0.002$) in ADG, ADFI, G:F, and d 21 BW was observed for pigs fed increasing phytase included in 0.09% aP diets (Table 2). When aP was increased from 0.09 to 0.27%, ADG, ADFI, G:F, and d 21 BW increased ($P < 0.001$) when no phytase was included in the diet, but when 1,000 FYT/kg of phytase was added, ADFI was increased ($P = 0.047$) without any change in ADG or G:F.

Pigs fed increasing levels of phytase within the 0.09% aP diet had a tendency ($P \leq 0.074$) for increased fibula and 10^{th} rib bone density and had increased (linear, $P = 0.019$) metacarpal bone density. There was a tendency ($P = 0.081$) for pigs fed 1,000 FYT/kg phytase added to the diet with 0.27% aP to have increased metacarpal bone density compared to pigs fed a 0.27% aP

diet with no added phytase. When aP was increased from 0.09 to 0.27% in diets without added phytase, bone density was increased ($P \leq 0.002$) in fibulas and metacarpals, and 10th rib bone density tended to be increased ($P = 0.084$). Comparing pigs fed diets containing either 0.09 or 0.27% aP with 1,000 FYT phytase, increasing the aP level also increased bone density ($P \leq 0.008$) in fibulas and metacarpals.

For all three bones, bone ash weight increased linearly ($P < 0.001$) when increasing phytase in diets with 0.09% aP as well as when 1,000 FYT/kg phytase was added in the diet containing 0.27% aP. For all three bones, there was an increase ($P < 0.001$) in bone ash weight when increasing aP from 0.09 to 0.27% when diets did not contain phytase. Comparing pigs fed diets containing either 0.09 or 0.27% aP with 1,000 FYT phytase, increasing the aP level also increased bone ash weight in all bones ($P < 0.001$).

Percentage bone ash tended to increase (linear, $P = 0.100$) in fibulas, and increased in metacarpal (linear, $P < 0.001$) and 10th rib (quadratic, $P = 0.004$) when increasing phytase in diets containing 0.09% aP. All bones showed an increase in percentage bone ash ($P \leq 0.038$) when adding 1,000 FYT/kg phytase in diets with 0.27% aP compared to pigs fed a 0.27% diet with no added phytase. Increasing aP from 0.09% to 0.27% in diets without added phytase increased percentage bone ash in all bones ($P \leq 0.074$). Comparing pigs fed diets containing either 0.09 or 0.27% aP with 1,000 FYT phytase, increasing the aP level also increased percentage bone ash in all bones ($P \leq 0.002$). Pigs fed 0.27% aP with no phytase had increased fibula and 10th rib % bone ash ($P = 0.018$, $P = 0.028$, respectively) compared to pigs fed diets with 0.09 aP and 1000 FYT/kg phytase.

Increasing phytase in diets containing 0.09% aP increased grams of Ca and P in all three bones (linear, $P \leq 0.027$). Grams of Ca and P was increased ($P \leq 0.023$) in fibulas and 10th ribs

and marginally increased ($P \leq 0.089$) in metacarpals when 1,000 FYT/kg was added to the diet containing 0.27% aP compared to pigs fed a 0.27% diet without added phytase. Increasing aP from 0.09% to 0.27% aP in diets without added phytase increased ($P \leq 0.007$) grams of Ca and P in fibulas and 10th ribs and increased ($P = 0.049$) P in metacarpals. Comparing pigs fed diets containing either 0.09 or 0.27% aP with 1,000 FYT phytase, increasing the aP level also increased ($P < 0.001$) grams of Ca and P in fibulas and 10th ribs and tended to increase ($P \leq 0.070$) Ca and P in metacarpals.

There was a quadratic response ($P \leq 0.034$) in metacarpals for both Ca and P percentage where pigs fed 600 FYT/kg phytase in the 0.09% aP diet had reduced Ca and P percentage. There was also higher percentage ($P = 0.044$) of Ca in metacarpals when pigs were fed a P deficient (0.09% aP) diet with no added phytase versus an adequate P (0.27% aP) diet when no phytase was added.

Using growth performance (ADG and G:F), aP release attributed to phytase was estimated to be 0.170% for 600 FYT/kg and 0.206% for 1,000 FYT/kg (Table 3). For bone density, 600 FYT/kg provided a 0.097% release and 1,000 FYT/kg a 0.110% aP release. For percentage bone ash, estimated aP release value for 600 FYT/kg was 0.142% and for 1,000 FYT/kg was 0.140% for the average of the three bones.

Discussion

Because of the limited availability of P within cereal grains and oilseeds, nutritionists often increase inorganic sources of P, such as monocalcium phosphate, to optimize growth and ensure normal bone formation. However, exogenous phytase is commonly utilized in diets to reduce diet costs, increase the digestibility of phytate-bound P, and decrease excess P in manure

(Simons et al., 1990; Selle and Ravindran, 2008; Li et al., 2013). Research has also shown that there are several beneficial effects when phytase is added to P deficient diets including improved digestibility of P, Ca, amino acids and energy and improved growth performance (Dersjant-Li et al., 2015; Arredondo et al. 2019).

Inadequate digestible P in swine diets can lead to restricted growth, increased incidence of lameness, and decreased bone P reserves (Buhler et al., 2010; She et al., 2017; Wensley et al., 2020b). It has been well documented that increasing phytase dose in P deficient diets improves both growth performance and bone characteristics (Gourley et al., 2018; Wensley et al., 2020b; Becker et al, 2021). With the extensive use of phytase in swine diets, there is a continuous effort to develop new phytase sources with improved functionality. Phytase sources can be categorized as 3- and 6-phytases, depending on the carbon site of hydrolysis (Augspurger et al., 2003). The phytase used in this study (HiPhorius 10; DSM Nutritional Products; Parsippany, NJ) is classified as a 6-phytase and is a modified *C. braakii* phytase expressed from a strain of *A. oryzae*.

Bones are routinely collected and analyzed because they are sensitive to changes in dietary P concentration. When P is not adequate, pigs are unable to maximize bone mineralization which can be measured using several analytical techniques such as bone ash or bone density. Percentage bone ash has become a common indicator of phytase efficacy (Gourley et al., 2018; Wensley et al., 2020b; Becker et al., 2021). Research suggests that conducting several different analyses on multiple bones may be more indicative of P status (Williams et al., 2022). Therefore, using multiple bones (ex. fibula, rib, and metacarpal) may provide a more robust estimate of aP release from phytase (Lee et al., 2021). Additionally, using multiple bone measurements in addition to percentage bone ash, such as bone density and bone P content, may

provide further insight on bone mineralization and sensitivity to dietary P and phytase inclusion. Research shows that multiple factors can impact the sensitivity of different bones to dietary P in swine diets and that different bones mineralize differently (Lee et al., 2021; Williams et al. 2022). Weight-bearing bones such as the metacarpal are prioritized for P reserves as compared to other bones such as ribs (Lee et al. 2021). In P deficiency, ribs would likely be mobilized first to ensure weight-bearing bones remain adequate in P and structurally sound (Crenshaw 1986). Due to these differences in bone mineralization and sensitivity to dietary P levels, we evaluated three different bones throughout the body to estimate the overall efficacy of the phytase in improving bone mineralization in the current study.

Several studies corroborate some of the findings observed in the current study. Thorsen et al. (2021) showed that in P deficient diets, the inclusion of HiPhorius phytase increased ADG, ADFI, bone breaking strength, and percentage bone ash. Like results of the current study, Thorsen et al. (2021) also observed a decrease in ADFI and ADG when feeding a P deficient diet with no added phytase compared to pigs fed diets with adequate P (NRC, 2012) using dicalcium phosphate. When adding phytase, increased growth performance in the later nursery stage was observed (d 14 to 42 after weaning) as well as a linear increase in percentage bone ash from femurs (Thorsen et al., 2021), which agrees with the linear increase in percentage bone ash for all three bones in pigs fed a 0.09% aP diet in the current study. To further confirm these findings, Zhai et al. (2023) observed increases in ADG, G:F, ATTD of P, and percentage bone ash in tibias when increasing HiPhorius phytase in nursery diets. The increase in bone mineralization, but no further improvement in growth performance seen in the current study for pigs fed 1,000 FYT/kg phytase as an addition to an adequate (0.27%) aP diet also agrees with past literature showing that increased P is needed to maximize bone characteristics relative to growth

(Cromwell et al., 1970; Augspurger et al., 2003; Wensley et al., 2020b). Furthermore, because of the response observed for increasing bone mineralization with added phytase in diets containing 0.27% aP, it could be argued that the P requirement is likely higher than 0.27% aP.

In our study, the magnitude of aP release at different FYT/kg inclusion rates depended on the response criteria measured; however, the calculated release values were similar to previous data (Zhai et al. 2023). The growth performance (using ADG and G:F) percentage aP release found by Zhai et al. (2023) was calculated to be 0.168% for 500 FYT/kg and 0.222% for 1,000 FYT/kg, and for percentage bone ash, aP release was 0.117% for 500 FYT/kg and 0.110% for 1,000 FYT/kg. In the current study, the growth performance percentage aP release was calculated to be 0.170% for 600 FYT/kg and 0.206% for 1,000 FYT/kg. For bone density, 600 FYT/kg provided a 0.097% release and 1,000 FYT/kg a 0.110% aP release. For percentage bone ash, estimated aP% release value for 600 FYT/kg was 0.142% and for 1,000 FYT/kg was 0.140% for the average of the three bones. The available P release values based on ADG and G:F were greater than those based on bone density or percentage bone ash measures, which is also in agreement with Zhai et al. (2023). This could be explained by the dependence of growth on P and the extra-phosphoric effect of phytase beyond P nutrition (Lu et al., 2019; Zhai et al., 2023). Phytase can increase the digestibility of other nutrients such as Ca, energy, and amino acids as well as P and therefore increase growth performance as a result (Dersjant-Li et al., 2015).

Ideally in phytase release curve studies, a standard curve has three points to determine the shape of the curve in relation to release estimation. This trial however was not truly designed to estimate release. Therefore, an assumption is being made that standard curve is linear. This is a reasonable assumption especially given the increased performance observed in pigs when adding

phytase the 0.27% aP diet, which indicates the requirement for aP is greater than 0.27%, thus it is fair to assume we were still in the linear portion of the curve with both treatments with 0.27% aP.

In conclusion, increasing phytase in diets formulated below the pig's P requirement improved growth performance and bone mineralization as expected. Increasing phytase in diets with 0.27% aP did not influence growth performance, but improved bone mineralization. This data confirms the efficacy of HiPhorius phytase and aP release values determined from different growth and bone measurement criteria.

References

Augspurger, N. I., D. M. Webel, X. G. Lei, and D. H. Baker. 2003. Efficacy of an E. coli phytase expressed in yeast for releasing phytate-bound phosphorus in young chicks and pigs. J. Anim. Sci. 81:474-473. doi:10.2527/2003.812474x.

Arredondo, M. A., G. A. Casas, and H. H. Stein. 2019. Increasing levels of microbial phytase increases the digestibility of energy and minerals in diets fed to pigs. Anim. Feed Sci. Technol. 248:27-36. doi:10.1016/j.anifeedsci.2019.01.001.

Becker, L. L., M. R. Wensley, J. M. DeRouchey, J. C. Woodworth, M. D. Tokach, R. D. Goodband and P. A. Lessard. 2021. Determining the phosphorus release of GraINzyme phytase in diets for nursery pigs. Transl. Anim. Sci. 5(3):txab105. doi:10.1093/tas/txab105.

Buhler, K., A. Liesegang, B. Bucher, C. Wenk, and J. Broz. 2010. Influence of benzoic acid and phytase in low-phosphorus diets on bone characteristics in growing-finishing pigs. J. Anim. Sci. 88(10):3363-3371. doi:10.2527/jas.2009-1940.

Crenshaw, T. D.1986. Reliability of dietary Ca and P levels and bone mineral content as predictors of bone mechanical properties at various time periods in growing swine. J. Nutr. 11:2155–2170.doi:10.1093/jn/116.11.2155.

Cromwell, G. L., V. W. Hays, C. H. Chaney, and J. R. Overfield. 1970. Effects of dietary phosphorus and calcium level on performance, bone mineralization and carcass characteristics of swine. J. Anim. Sci. 30:519-525. doi:10.2527/jas1970.304519x.

Cromwell, G. L., T. S. Stahly, R. D. Coffey, H. J. Monegue, and J. H. Randolph. 1993. Efficacy of phytase in improving bioavailability of phosphorus in soybean meal and corn-soybean meal diets for pigs. J. Anim. Sci. 71:1831-1840. doi:10.2527/1993.7171831.

Dersjant-Li, Y., A. Awati, H. Schulze, and G. Partridge. 2015. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. J. Sci. Food Agric. 95(5):878-896. doi:10.1002/jsfa.6998.

Eeckhout, W., and M. De Paepe. 1994. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Anim. Feed Sci. Technol. 47:19-29. doi:10.1016/0377-8401(94)90156-2.

Gonzalez-Vega, J. C., Y. Liu, J. C. McCann, C. L. Walk, J. J. Loor, and H. H. Stein. 2016. Requirement for digestible calcium by eleven- to twenty-five-kilogram pigs as determined by growth performance, bone ash concentration, calcium and phosphorus balances, and expression of genes involved in transport of calcium in intestinal and kidney cells. J. Anim. Sci. 94:3321-3334. doi:10.2527/jas.2016-0444.

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.

Igbasan, F. A., K. Männer, G. Miksch, R. Borriss, A. Farouk, and O. Simon. 2000. Comparative studies on the in vitro properties of phytases from various microbial origins. Archiv für Tierernaehrung. 53(4), 353-373. doi:10.1080/17450390009381958

Lautrou, M., C. Pomar, J. Y. Dourmad, A. Narcy, P. Schmidely, and M. P. Léourneau-Momtminy. 2020. Phosphorus and calcium requirements for bone mineralisation of growing pigs predicted by mechanistic modeling. Animal. S2:s313-s322. doi:10.1017/S1751731120001627.

Lee, S. A., L. V. Lagos, M. R. Bedford, and H. H. Stein. 2021. Quantities of ash, Ca, and P in metacarpals, metatarsals, and tibia are better correlated with total body bone ash in growing pigs than ash, Ca, and P in other bones. J. Anim. Sci. 99:1-6. doi:10.1093/jas/skab149.

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(9):3898-3906. doi:10.1093/jas/skz232.

NRC. 1998. Nutrient Requirements of Swine, 10th ed. Natl. Acad. Press, Washington D.C.

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

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

RStudio Team, 2020. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA.

Selle, P. H., and V. Ravindran. 2008. Phytate-degrading enzymes in pig nutrition. Livest. Sci. 113:99-122. doi:10.1016/j.livsci.2009.01.006.

Selle, P. H., A. J. Coweison, V. Ravindran. 2009: Consequences of calcium interactions with phytate and phytase for poultry and pigs. Liv. Sci. 124:126-141. doi:10.1016/j.livsci.2009.01.006.

Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. A. Kemme, P. B. Slump, K. D. Bos, M.G.E. Wolters, R. F. Beudeker, G. J. Verschoor. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Brit. J. Nutr. 64:525–540. doi:abs10.1080/09712119.2000.9706294.

She, Y., Y. Liu, J. C. Gonzalez-Vega, and H. H. Stein. 2017. Effects of graded levels of an Escherichia coli phytase on growth performance, apparent total tract digestibility of phosphorus, and on bone parameters of weanling pigs fed phosphorus-deficient corn-soybean meal-based diets. Anim. Feed Sci. Technol. 232:102-109. doi:10.1016/j.anifeedsci.2017.08.005.

Thorsen, M., Nielsen, L. A., Zhai, H. X., Zhang, Q., Wulf-Andersen, L., and Skov, L. K. 2021. Safety and efficacy profile of a phytase produced by fermentation and used as a feed additive. Heliyon. 7(6):e07237. doi:10.1016/j.heliyon.2021.e07237.

Wensley, M. R., C. M. Vier, J. T. Gebhardt, M. D. Tokach, J. C. Woodworth, R. D. Goodband, and J. M. DeRouchey. 2020a. Technical Note: Assessment of two methods for estimating bone ash in pigs. J. Anim. Sci. 98:1-8. doi:10.1093/jas/skaa251.

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., T. Chin, J. T. Gebhardt, M. D. Tokach, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, J. R. Bergstrom, M. Rahe, C. Siepker, and P. Sitthicharoenchai. 2022. Abstract: The effect of different bone and analytical method on assessment of bone mineralization response to dietary P, phytase, and vitamin D in nursery pigs. J. Anim. Sci. 100:25-25. doi:10.1093/jas/skac064.040.

Zhai, H., J. Zhang, Z. Wang, S. Wang, S. Prasad, K. Stamatopoulos, and S. Duval. 2023. Comparison of digestible and available phosphorus release values for a novel phytase determined with fecal phosphorus digestibility and bone mineralization in weaner pigs. Anim. Feed Sci. Technol. 115580. doi:10.1016/j.anifeedsci.2023.115580.

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

aP, %:	0.09			0.27	
Phytase, FYT/kg:	0	600	1,000	0	1,000
Ingredient, %					
Corn	63.26	63.26	63.26	63.26	63.26
Soybean meal	33.65	33.65	33.65	33.65	33.65
Limestone	0.26	0.26	0.26	0.44	0.44
Monocalcium P	0.06	0.06	0.06	0.91	0.91
Salt	0.60	0.60	0.60	0.60	0.60
L-Lys-HCl	0.30	0.30	0.30	0.30	0.30
DL-Met	0.12	0.12	0.12	0.12	0.12
L-Thr	0.12	0.12	0.12	0.12	0.12
L-Val	0.01	0.01	0.01	0.01	0.01
Trace mineral[2] premix	0.15	0.15	0.15	0.15	0.15
Vitamin premix[3]	0.25	0.25	0.25	0.25	0.25
Sand[4]	1.23	1.22	1.22	0.19	0.18
Phytase[5]	---	0.006	0.010	---	0.010
Calculated analysis					
Standard ileal digestible (SID) amino acids, %					
Lys	1.24	1.24	1.24	1.24	1.24
Ile:Lys	63	63	63	63	63
Leu:Lys	129	129	129	129	129
Met:Lys	33	33	33	33	33
Met and Cys:Lys	57	57	57	57	57
Thr:Lys	63	63	63	63	63
Trp:Lys	18.7	18.7	18.7	18.7	18.7
Val:Lys	69	69	69	69	69
His:Lys	41	41	41	41	41
Total Lys, %	1.39	1.39	1.39	1.39	1.39
NE, kcal/kg	2,407	2,407	2,407	2,407	2,407
SID Lys:NE, g/Mcal	5.14	5.14	5.14	5.14	5.14
aP, %	0.09	0.09	0.09	0.27	0.27
Ca, %	0.43	0.43	0.43	0.64	0.64
P, %	0.36	0.36	0.36	0.54	0.54
Ca:P ratio	1.20	1.20	1.20	1.20	1.20
Analyzed composition[6]					
Ca, %	0.36	0.46	0.36	0.68	0.59
P, %	0.36	0.38	0.41	0.57	0.55
Ca:P ratio	0.98	1.19	0.89	1.19	1.07

[1]Diets were fed for 21 d from approximately 9 to 18 kg.

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

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

[4]To form the treatments, a hand-add of sand, limestone, monocalcium P, and phytase was used to equalize the dietary contribution of all other ingredients.

[5]HiPhorius 10 2400 phytase was analyzed to contain 11,914 FYT/g and was provided by DSM Nutritional Products, Parsippany, NJ.

[6]Complete diet samples were taken during the bagging of experimental diets with a sub-sample collected from every third bag and pooled into one homogenized sample per dietary treatment. After homogenization, samples were stored at -20^{o}C until analysis (KSU Soils Lab in Manhattan, KS) for Ca and P. Two samples of each dietary treatment were analyzed in duplicate to obtain average Ca and P values.

Table 3.2. Effects of HiPhorius phytase on growth performance and bone mineralization in nursery pigs[1]

							P =				
aP, %:	0.09			0.27			Phytase in 0.09% aP		Phytase in 0.27% aP	P level in 0 FYT/kg	P level in 1,000 FYT/kg
Phytase, FYT/kg:	0	600	1,000	0	1,000	SEM	Linear	Quadratic			
BW, kg											
d 0	8.6	8.7	8.7	8.6	8.6	0.18	0.990	0.985	0.999	0.999	0.998
d 21	17.0	19.3	20.0	20.3	20.7	0.41	< 0.001	0.227	0.903	< 0.001	0.457
d 0 to 21											
ADG, kg	0.40	0.51	0.53	0.55	0.57	0.014	< 0.001	0.051	0.754	< 0.001	0.153
ADFI, kg	0.67	0.75	0.76	0.80	0.83	0.021	0.002	0.184	0.779	< 0.001	0.047
G:F	0.59	0.67	0.70	0.68	0.69	0.010	< 0.001	0.094	0.997	< 0.001	0.973
Bone density, g/mL											
Fibula	1.17	1.23	1.21	1.27	1.30	0.171	0.068	0.156	0.812	0.001	0.008
Metacarpal	1.16	1.17	1.18	1.20	1.23	0.008	0.019	0.419	0.081	0.002	0.002
10th Rib	1.27	1.35	1.37	1.41	1.46	0.037	0.074	0.606	0.871	0.084	0.413
Bone ash, g											
Fibula	0.41	0.67	0.66	0.72	1.00	0.044	< 0.001	0.057	< 0.001	< 0.001	< 0.001
Metacarpal	0.70	0.95	1.04	1.12	1.34	0.046	< 0.001	0.405	0.006	< 0.001	< 0.001
10th Rib	0.48	0.77	0.86	1.00	1.31	0.056	< 0.001	0.370	0.003	< 0.001	< 0.001
Bone ash, %											
Fibula[2]	38.3	43.8	42.5	45.6	53.7	0.02	0.100	0.229	0.038	0.074	0.002
Metacarpal	30.1	34.0	35.3	36.3	40.4	0.01	< 0.001	0.384	0.001	< 0.001	< 0.001
10th Rib[3]	44.3	49.1	48.7	51.2	54.3	0.01	< 0.001	0.004	0.004	< 0.001	< 0.001
Bone Ca, g											
Fibula	0.17	0.27	0.28	0.28	0.43	0.022	< 0.001	0.212	< 0.001	0.007	< 0.001
Metacarpal	0.30	0.32	0.38	0.29	0.47	0.024	0.027	0.343	0.089	0.110	0.070
10th Rib	0.18	0.29	0.33	0.39	0.51	0.028	< 0.001	0.526	0.023	< 0.001	< 0.001
Bone Ca, %											
Fibula	41.1	40.5	43.0	38.3	41.5	1.95	0.528	0.458	0.742	0.837	0.983
Metacarpal	41.3	33.9	36.8	34.8	35.5	1.60	0.027	0.020	0.998	0.044	0.979
10th Rib	37.0	37.6	37.5	38.5	38.8	1.46	0.756	0.877	0.999	0.929	0.958

Bone P, g											
Fibula	0.07	0.12	0.13	0.13	0.20	0.01	< 0.001	0.189	< 0.001	0.003	< 0.001
Metacarpal	0.13	0.15	0.18	0.18	0.22	0.01	0.011	0.388	0.075	0.049	0.059
10th Rib	0.09	0.15	0.17	0.20	0.26	0.01	< 0.001	0.472	0.020	< 0.001	< 0.001
Bone P, %											
Fibula	17.8	18.3	19.5	17.5	18.9	0.91	0.204	0.619	0.773	0.998	0.989
Metacarpal	18.4	15.6	17.1	16.1	16.6	0.75	0.140	0.034	0.994	0.218	0.986
10th Rib	18.2	19.1	18.9	19.5	19.7	0.74	0.391	0.614	0.999	0.626	0.921

[1]A total of 297 pigs (DNA 241 x 600; initially 8.64 ± 0.181 kg) were used in a 21-d growth study. There were 5 pigs per pen and 12 pens per treatment. HiPhorius 10 2400 phytase was provided by DSM Nutritional Products (Parsippany, NJ). One pig per pen was euthanized and the right fibula, 10th rib, and metacarpal were collected to determine bone density, bone ash, and bone Ca and P.

[2]Pigs fed 0.27% aP with no phytase had increased (P = 0.018) fibula % bone ash compared to pigs fed 0.09 aP and 1000 FYT/kg phytase.

[3]Pigs fed 0.27% aP with no phytase had increased (P = 0.028) 10th rib % bone ash compared to pigs fed 0.09 aP and 1000 FYT/kg phytase.

Table 3.3. Estimated aP % release of HiPhorius phytase[1]

	FYT/kg	
Response	600	1,000
ADG	0.154	0.180
G:F	0.187	0.233
Average of growth performance	0.170	0.206
Bone density		
Fibula	0.123	0.082
Metacarpal	0.051	0.103
10th rib	0.117	0.147
Average of 3 bones	0.097	0.110
Percentage bone ash		
Average of 3 bones	0.142	0.140
Fibula	0.155	0.118
Metacarpal	0.129	0.172
10th rib	0.143	0.131
Average of 3 bones	0.142	0.140
Average of all bone measures	0.120	0.125

[1]Release estimates were calculated using the aP levels of the 0.09% and 0.27% aP diets to develop a standard curve with estimated marginal intake of aP above the diet with 0.09% aP as the independent variable and response as the dependent variable. Response criteria including growth and measures of bone mineralization for the 0.09% aP + 600 FYT/kg and 0.09% aP + 1,000 FYT/kg treatment groups were then used to estimate the marginal aP intake, and thus estimate the aP release for each phytase dose for each response criteria.