Evaluation of phosphorus requirement and calcium to phosphorus ratio in nursery and finishing pigs and stability of phytases

by

Carine Mirela Vier

D.V.M., Federal University of Rio Grande do Sul, 2016

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine/Pathobiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2019

Abstract

This dissertation consisted of 6 chapters involving studies with standardized total tract digestible (STTD) phosphorus (P) requirements of nursery and finishing pigs, dietary calcium (Ca) to P ratio, economic model for optimum P level, stability of phytases, and reproducibility of research results. Chapter 1 describes 2 experiments that evaluated the STTD P requirements of nursery pigs fed diets without or with 1,000 phytase units (FYT). These data provided empirical evidence that for 11- to 23-kg pigs, the NRC (2012) accurately estimates the STTD P requirement on a g/d basis. As a percentage of the diet, the STTD P requirement for diets without or with 1,000 FYT added phytase ranged from 0.34 to 0.42% to maximize average daily gain (ADG) and gain:feed ratio (G:F). Chapter 2 characterized a dose response to increasing STTD P concentration in diets for 24- to 130-kg pigs. The digestible P requirements to maximize ADG and G:F were 122 and 116% of NRC (2012) estimates across dietary phases, respectively. A greater STTD P of 131% of NRC (2012) estimates, was required to optimize bone mineralization. The third chapter consisted of two experiments to determine the effects of Ca:P ratio in diets adequate in STTD P on performance of 26- to 127-kg pigs fed diets without or with 1,000 FYT added phytase. The maximum responses in ADG, hot carcass weight, and bone ash were estimated at 1.63:1, 1.11:1 to 1.60:1, and 1.25:1 analyzed Ca:P and at 1.75:1, 1.28:1 to 1.71:1, and 1.40:1 STTD Ca:STTD P, respectively. Moreover, expressing ADG on a STTD Ca:STTD P basis provided a more consistent estimate of the ideal Ca:P ratio among the two studies than analyzed Ca to analyzed P ratio. The study presented in chapter 4 described a Microsoft Excel®-based P economic tool. This tool was developed based on the information generated in the above chapters. The objective of the tool is to contrast current dietary STTD P concentrations to recommended values that yield maximum growth performance while accounting for financial implications over different

scenarios. In chapter 5, an experiment was conducted to evaluate the effects of storing three commercially available phytase products over 90 d under high temperature and high humidity conditions on phytase stability, growth performance, bone mineralization, and serum myo-inositol concentration of nursery pigs. Residual phytase activity decreased as storage time increased, and when phytases were stored in a vitamin and trace mineral premix compared to pure form. Except for HiPhos in pure form, bone ash was reduced when phytases were stored for 90 d compared to a positive control diet with no added phytase. Finally, chapter 6 focuses on reproducibility of research results in the animal sciences from the aspects of making the raw data available, documenting the statistical model, and reporting that is integrated with the statistical analysis. Several reproducible research tools are presented to make data and code publicly accessible in a data repository, and to generate dynamic reports that accurately describe the steps involved in generating the research findings.

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Acknowledgements

The successful completion of this dissertation required the dedication and effort of so many people, and I would like to acknowledge those who have contributed to make it possible. I would like to express my sincerest gratitude to the K-State Applied Swine Nutrition team for the opportunity to further my education. This was a life changing experience and I can't thank them enough for that. I'd like to thank my major advisor, Dr. Dritz, who inspired me to be better, encouraged me to work harder, and pushed me towards my highest potential. I am particularly grateful for his kindness, friendship, and endless support. I have also been blessed with the guidance of Drs. Tokach, Woodworth, DeRouchey, and Goodband. Their knowledge, dedication, training, and mentorship were essential for my personal and professional development. Thank you to Dr. Hefley for serving on my committee and assisting in my preliminary exam.

To my fellow graduate students (current and past), thank you for making this graduate experience unforgettable. I am extremely honored to be part of the applied swine nutrition team alongside them, and am grateful for the generous help, for the friendship, and for the moments we shared. A special thanks to Mariana for being the best office mate and friend in this journey. I am proud to have met so many amazing people that I know are going to be leaders in the swine industry, and I look forward to continuing to work with them in the near future.

I'd like to recognize Theresa Rathbun and all the laboratory student workers; their assistance was indispensable. Thank you to all the farm workers, feed mill employees, undergraduate students, office staff, and others who have helped me over the years. I would also like to thank New Horizon Farms (Pipestone, MN) for providing the animals and research facilities to conduct part of the trials, and to Marty Heintz, Allan Morris, Heath Houselog, and

Whitney Adler for technical assistance. I wish to acknowledge the extensive funding and research support that made this program possible: Genus PIC and DSM Nutritional Products.

I would also like to thank Júlia, Carla, Leo, Mari, Henrique, Márcio, Gabi, Andy, Amanda, Leandro, Isa, David, Arkin, Michelle, Fe, Jamil for their friendship throughout these years. I will always cherish the moments we shared the most! This experience wouldn't be near as much fun and enjoyable without you all. Thank you to Andrew for his support, love, uplifting thoughts, and words of encouragement during this journey.

Lastly, my greatest thanks is to my parents, Vanice and Marcelino, who are my absolute everything. I know the many sacrifices they have done to enable me to pursue my dreams, no matter how big and far from home these dreams were. They are my inspiration, and their selfless support and love is beyond price.

Dedication

This dissertation is dedicated to my parents, Vanice and Marcelino, to my grandmothers, Romilda Vier and Ilse Lehnen, and in loving memory of Victor Lehnen.

Preface

This dissertation is original work completed by the author, C. M. Vier. All chapters were formatted for publication according to the required standards of the Journal of Animal Science.

Chapter 1 - Effects of standardized total tract digestible phosphorus on growth performance of 11- to 23-kg pigs fed diets with or without phytase

ABSTRACT

Two experiments were conducted to determine the standardized total tract digestible phosphorus (**STTD P**) requirement for 11- to 23-kg nursery pigs fed diets with or without phytase. A total of 1,080 and 2,140 pigs (PIC 359 × Camborough, Hendersonville, TN; initially 11.4 ± 0.29 and 11.1 ± 0.24 kg) were used in Exp. 1 and 2, respectively. There were 23 to 27 pigs per pen with 6 and 12 replicate pens per treatment in Exp. 1 and Exp. 2, respectively. After weaning, pigs were fed a common pelleted diet with 0.45% STTD P for 7 d, and a common phase 2 meal diet with 0.40% STTD P for 14 d in Exp. 1 and 18 d in Exp. 2. Pens of pigs were then were allotted to dietary treatments in a randomized complete block design with body weight (**BW**) as the blocking factor. In Exp. 1, dietary treatments consisted of 0.26, 0.30, 0.33, 0.38, 0.43, 0.48 and 0.53% STTD P. Treatments were achieved with the inclusion of monocalcium phosphate at the expense of corn. In Exp. 2, diets contained 1,000 phytase units (**FYT;** Ronozyme Hiphos 2500, DSM Nutritional Products, Inc., Parsippany, NJ) with assumed release value 0.132% STTD P, and treatments consisted of 0.30, 0.33, 0.38, 0.43, 0.48, 0.53 and 0.58% STTD P. These STTD P concentrations included the expected phytase release of 0.132% STTD P. In both experiments, a similar 1.17:1 Ca:P ratio was maintained across treatments. Statistical models included linear model (**LM),** quadratic polynomial (**QP**), broken-line linear (**BLL**), and broken-line quadratic (**BLQ**). In Exp. 1, increasing STTD P increased (linear, $P < 0.001$) ADG, ADFI, G:F, final BW, grams of STTD P intake per day and per kilogram of gain. There also was

a marginal quadratic response for G:F ($P < 0.066$). In Exp. 2, ADG and G:F increased quadratically ($P < 0.05$), while ADFI increased linearly ($P = 0.060$) with increasing STTD P. The BLL and QP model provided similar fit to G:F in Exp. 1, estimating the requirement for maximum G:F at 0.34 and 0.42%, respectively. The BLL was the best fitting model for ADG and G:F in Exp. 2, estimating the breakpoint at 0.40 and 0.37% STTD P, respectively. The BLL and BLQ models estimated the breakpoint for ADG as a function of STTD P intake in g/d at 2.92 and 3.02 g/d, respectively. These data provide empirical evidence that for 11- to 23-kg pigs, the NRC (2012) accurately estimates the STTD P requirement on a g/d basis. As a percentage of the diet, the STTD P requirement for diets without or with 1,000 FYT added phytase ranged from 0.34 to 0.42%.

Key words: growth, nursery pigs, modeling, phosphorus requirement, phytase

INTRODUCTION

Phosphorus (**P**) is the second most abundant mineral in the body after calcium (**Ca**) and is required for multiple biological functions (Berndt and Kumar, 2009). However, P concentration can greatly impact dietary cost as P is considered the third most expensive nutrient in swine diets. Thus, driven by economic and environmental concerns, P supplementation is typically associated with lower safety margins in swine diets compared to Ca (Crenshaw, 2001).

The NRC (2012) reports the P requirement estimates by pigs on a standardized total tract digestible (**STTD**) basis. The requirement estimates of STTD P for pigs weighing less than 20 kg of BW, however, were derived from a simple mathematical regression model that includes a limited number of published empirical studies. There is a need for more empirical data to validate the NRC estimates. In fact, recent research suggests that the NRC (2012) requirement estimates may underestimate the P concentration needed to optimize pig growth performance

(Zhai and Adeola, 2013, Adeola et al., 2015, Wu et al., 2018). Thus, it is important to reassess the STTD P requirement of growing pigs in commercial pig production.

Approximately 60 to 80% of P in feedstuffs of plant origin is stored in phytic acid (Eeckhout and Paepe, 1994). Pigs poorly utilize the phytate-bound-P because they lack sufficient endogenous phytase to effectively cleave the phosphates from the phytate. Thus, practical nursery diets are typically formulated with added phytase to increase P availability to the pig while decreasing the need for expensive inorganic sources of P (Selle and Ravindran, 2008).

We hypothesized that the STTD P requirements would be similar for pigs fed diets with and without the inclusion of phytase given the P release values from the phytase are correct. To our knowledge, empirical data determining the STTD P requirement of nursery pigs with and without phytase is limited. Therefore, the objective of our study was to determine the effects of increasing STTD P concentration while maintaining a similar Ca:P ratio in diets with or without phytase (1,000 phytase units; **FYT**) on growth performance of 11- to 23-kg pigs housed under commercial conditions.

MATERIAL AND METHODS

The Kansas State University Institutional Animal Care and Use Committee (Manhattan, KS) approved all experimental procedures in this study.

Animals and Diets

Two studies were conducted at a commercial research-nursery site in southwestern Minnesota (New Horizon Farms, Pipestone, MN). The facility was environmentally controlled and mechanically ventilated. Two rooms were used, each containing 42 pens (3.70×2.30 m^2) with completely slatted flooring and a deep pit for manure storage. Each pen was equipped with a 5-hole stainless steel dry self-feeder (SDI Industries, Alexandria, SD) and a pan waterer. The

facilities were equipped with a computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN) capable of blending and distributing diets to each pen as specified. Furthermore, the system can measure and record daily feed additions to individual pens. At placement in the nursery, barrows and gilts (PIC 359 × Camborough, Genus PIC, Hendersonville, TN) were balanced by sex and allowed ad libitum access to feed and water throughout the experiments.

A total of 1,080 pigs (initial average BW of 11.4 ± 0.29 kg) in Exp. 1 and 2,140 pigs (initial average BW of 11.1 ± 0.24 kg) in Exp. 2 were used in two 21-d growth trials. Pigs in Exp. 1 were weaned at approximately 21 d of age and pigs in Exp. 2 were weaned at approximately 19 d of age. A common phase 1 pelleted diet was fed for 7 d in both trials, and a common phase 2 meal diet was fed for 14 or 18 d in Exp. 1 and Exp. 2, respectively. Both common diets were formulated to be at the pigs' STTD P requirement based on the NRC estimates (0.45 and 0.40% STTD P, respectively). At d 0 of the trial, pens of pigs were allotted to dietary treatments in a randomized complete block design with BW as the blocking factor. There were 6 replicate pens per treatment with 23 to 27 pigs (similar numbers of barrows and gilts) per pen in Exp. 1, and 12 replicate pens per treatment with 24 to 27 pigs (similar numbers of barrows and gilts) per pen in Exp. 2.

All treatment diets were manufactured at the New Horizon Farms Feed Mill in Pipestone, MN and fed in meal form. In Exp. 1, two experimental corn-soybean meal–based diets were formulated (Table 1) to contain 0.26 and 0.53% STTD P and then were blended using the robotic feeding system to create the intermediate STTD P levels. The STTD P levels were achieved by increasing the addition of limestone and monocalcium phosphate at the expense of corn, with no added phytase. The NRC (2012) suggested a total Ca:P ratio between 1.10 and 1.25:1. Therefore, a similar 1.17:1 to 1.18:1 total Ca:P ratio was maintained across dietary treatments. The

percentage of low and high STTD P diet blended to create the treatment diets were 100:0, 88:12, 75:25, 56:44, 37:63, 19:81, and 0:100 to achieve 0.26, 0.30, 0.33, 0.38, 0.43, 0.48, and 0.53% STTD P, respectively. The NRC (2012) requirement estimate for nursery pigs from 11- to 23-kg, expressed as a percentage of the diet, is 0.33% STTD P. Therefore, treatment concentrations represented 80, 90, 100, 115, 130, 145, and 160% of the NRC requirement estimate.

In Exp. 2, two experimental corn-soybean meal–based diets were formulated (Table 1) to contain 0.30 and 0.58% STTD P and then were blended using the robotic feeding system to create the intermediate STTD P levels. The diets contained 1,000 FYT of Ronozyme Hiphos 2500 (DSM Nutritional Products, Inc., Parsippany, NJ) with assumed release values of 0.15% available P and 0.132% STTD P. The STTD P levels were achieved by increasing the amount of limestone and monocalcium phosphate at the expense of corn. A similar 1.17:1 Ca:P ratio was maintained across dietary treatments. The percentage of low and high STTD P diet blended to create the treatment diets were 100:0, 89:11, 71:29, 53:47, 36:64, 18:82, and 0:100 to achieve 0.30, 0.33, 0.38, 0.43, 0.48, 0.53, and 0.58% STTD P, respectively. These STTD P concentrations included the expected phytase release of 0.132% STTD P. The treatment concentrations represented 90, 100, 115, 130, 145, 160 and 175% of the NRC requirement. The lowest STTD P diet did not contain any monocalcium phosphate. Thus, the STTD P was entirely from corn, soybean meal, and the P liberated by phytase.

Pigs were weighed and feed disappearance was measured on d 0 and 21 in both experiments to determine ADG, ADFI, G:F ratio, grams of STTD P intake per day, and grams of STTD P intake per kilogram of gain. The STTD P, based on formulated values, were multiplied by ADFI to calculate grams of STTD P intake per day. The total grams of STTD P intake, based

on formulated values, were divided by total BW gain to calculate the grams of STTD P intake per kilogram of gain.

Chemical Analysis

Representative diet samples were obtained from all feeders of each treatment and delivered to the Kansas State University Swine Laboratory, Manhattan, KS, and stored at -20°C until analysis. Samples of the diets were combined within dietary treatment, and a composite sample from each treatment was analyzed in duplicate (Ward Laboratories, Inc., Kearney, NE). Samples were analyzed for DM (method 935.29; AOAC International, 1990), CP (method 990.03; AOAC International, 1990), Ca (method 985.01; AOAC International, 1990), P (method 985.01; AOAC International, 1990), ash (method 942.05, AOAC International, 1990), and ether extract (method 969.10, AOAC International, 1990). In Exp. 2, a composite sample of the low (0.30% STTD P) and high (0.58% STTD P) diets was analyzed for phytase activity (method 300.24; AOAC International, 2009) in duplicate (New Jersey Feed Laboratory Inc., Trenton, NJ).

Statistical Analysis

Data from both experiments were analyzed as a randomized complete block design with block as a random effect and pen as the experimental unit. Polynomial contrasts were implemented to evaluate the functional form of the dose response to increasing dietary STTD P on ADG, ADFI, G:F, BW, grams of STTD P intake per day, and grams of STTD P intake per kilogram of gain. The coefficients for the unequally spaced linear and quadratic contrasts were derived using the IML procedure in SAS (Version 9.3, SAS Institute Inc., Cary, NC). Statistical models were fit using GLIMMIX procedure of SAS. Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 \leq P \leq 0.10$.

In addition, the effects of the STTD P levels on ADG and G:F were fit using procedures outlined by Gonçalves et al. (2016). Briefly, models were expanded to account for heterogeneous residual variances when needed. Competing statistical models included a linear (**LM**), quadratic polynomial (**QP**), broken-line linear (**BLL**), and broken-line quadratic (**BLQ**). Dose response models were compared based on the Bayesian information criterion (**BIC**), where the smaller the value, the better (Milliken and Johnson, 2009). A decrease in BIC greater than 2 was considered a significant improvement in model fit. The 95% confidence interval of the estimated requirement to reach maximum performance was computed. Results reported correspond to inferences yielded by the best fitting models.

RESULTS

Chemical Analysis

The analyzed DM, CP, ether extract, ash, Ca, and P were consistent with formulated values (Tables 2 and 3). In both experiments, average values of analyzed P were approximately 7% lower than formulated values, which is still below the acceptable analytical variation (AAFCO, 2015). Analyzed P content increased with increasing STTD P treatments. Average values of analyzed Ca were approximately 7 and 15% higher than formulated values in Exp. 1 and Exp. 2, respectively. Chemical analysis of dietary Ca is typically more variable than P and the Ca analytical variability observed in this study is still within the acceptable variation based on AAFCO (2015). Moreover, they followed a stepwise increase as expected with the designed treatment structure. In Exp. 2, the analyzed phytase activity in the low (0.30% STTD P) and the high (0.58% STTD P) diets were 1,760 and 1,755 FYT/kg, respectively. Although the values of analyzed phytase activity were higher than formulated values, the resulting STTD P release according to the manufacturer would only represent an increase from 0.132 to 0.150%. In

addition, the variability in the phytase analysis of complete diets is typically greater than the phytase analysis of pure products (Kim and Lei, 2005).

Experiment 1

Average daily gain, ADFI, and G:F increased (linear, $P < 0.05$; Table 4) with increasing STTD P. There also was a marginal response (quadratic, $P < 0.066$) for G:F, with the greatest improvement in G:F as STTD P increased from 0.26 to 0.33%. There was a significant linear effect ($P = 0.001$) of increasing STTD P on final BW. The greatest improvement in final BW, however, was observed at 0.43% STTD P. Grams of STTD P intake per day and grams of STTD P intake per kilogram of gain increased (linear, $P = 0.001$) with increasing levels of STTD P.

The responses for ADG and ADFI were not modeled due to their linear nature. Heterogeneous variance was used for feed efficiency models. Feed efficiency had similar fitting models for the BLL and QP (Figure 1). The BLL breakpoint for G:F was estimated at 0.34% (95% CI: [0.30, 0.37%]) STTD P and the regression equation was:

G:F, g/kg = 696.63 – 427.26 × (0.3358 – STTD P) if STTD P < 0.34%,

G:F, g/kg = 696.63 if STTD P ≥ 0.34%

For the QP model, the maximum G:F was estimated at 0.42% (95% CI: [0.36, > 0.53%]) STTD P, with 99% of maximum performance being achieved with 0.36% STTD P. The regression equation for the QP model was:

G:F, g/kg = 456.59 + 1107.49 × (STTD P) – 1307.16 × $(\text{STTD P})^2$

Experiment 2

Increasing STTD P improved (quadratic, $P < 0.05$) ADG and G:F (Table 5). The greatest improvement was observed as the STTD P increased from 0.30 to 0.43% for ADG, and from 0.30 to 0.38% for G:F, with no improvements thereafter. Average daily feed intake increased

(linear, $P = 0.060$) with increasing the STTD P, with the highest feed intake observed at 0.48% STTD P. There was a significant linear response ($P = 0.028$) in final BW. The heaviest final weight, however, was observed at 0.43% STTD P. Also, grams of STTD P intake per day and grams of STTD P intake per kilogram of gain increased (linear, $P < 0.001$) with increasing levels of STTD P.

The response for ADFI was not modeled due to its linear nature. Homogeneous variance was used for ADG models and heterogeneous variance was used for feed efficiency models. The best fitting model was the BLL for ADG and G:F. The BLL breakpoint for ADG was estimated at 0.40% (95% CI: [0.33, 0.47]%) STTD P (Figure 2). Based on the best fitting model, the estimated regression equation was:

ADG, g = 543.97 – 289.79 × (0.3993 – STTD P) if STTD P < 0.40%,

ADG, g = 543.97 if STTD P ≥ 0.40%

For G:F, the breakpoint was estimated at 0.37% (95% CI: [0.29,0.45]%) STTD P, and the regression equation for the BLL model (Figure 3) was:

G:F, g/kg = 711.76 – 301.08 × (0.37 – STTD P) if STTD P < 0.37%,

G:F, g/kg = 711.76 if STTD P ≥ 0.37%

The ADG was also modeled as a function of STTD P intake in grams per day. The BLL and BLQ models has similar fit (Figure 4). The BLL breakpoint was estimated at 2.92 g/d (95% CI: [2.56, 3.27g/d]) STTD P and the regression equation was:

ADG, g = 545.11 – 51.3991 × (2.917 – STTD P in g/d) if STTD P intake < 2.92 g/d,

ADG, g = 545.11 if STTD P intake ≥ 2.92 g/d.

The BLQ breakpoint was estimated at 3.02 g/d (95% CI: [3.00, 3.03g/d]) STTD P. The regression equation for the BLQ model was:

ADG, g = 544.96 – 17.2077 × (3.019 – STTD P in g/d) – 35.7972 × (3.019 – STTD P in g/d)^2 if STTD P intake < 3.02 g/d,

ADG, g = 544.96 if STTD P intake ≥ 3.02 g/d.

DISCUSSION

In 2012, the NRC started to express the P requirement estimate by pigs on a STTD basis. The STTD P measures the digestible P utilization while accounting for the basal endogenous losses. The STTD P can be utilized in diet formulation as it is additive in mixed diets fed to pigs (NRC, 2012). The current study was designed to provide more information of the STTD P requirement of nursery pigs.

Limited research has evaluated the STTD P requirement of nursery pigs. Recent research conducted by Wu et al., (2018) determined the P requirement for growth performance of weaned pigs from 6- to 13-kg pigs when offered diets formulated to contain graded levels of STTD P that ranged from 80 to 140% of NRC on a diet concentration basis. Similar to our findings, higher STTD P estimates than the NRC (2012) requirement estimates were observed. For ADG, the BLL model estimated the requirement as 91% of the NRC (2012) while the more sensitive QP model resulted in a higher requirement estimate of 117% of NRC (2012). Depending on the statistical model, the estimated STTD P requirement for maximum feed efficiency ranged from 102 to greater than 140% of NRC (2012). The NRC (2012) estimated the STTD P requirement for 11- to 23-kg pigs at 0.33% of the diet. It is important to acknowledge that the NRC estimates the STTD P requirement of nursery pigs weighing less than 20 kg BW using a simple mathematical regression approach. Therefore, the requirement for STTD P as a percentage of the diet is related to the animal's BW as follows:

STTD P requirements (% of diet) = 0.6418 – 0.1083 × ln(BW)

Only two empirical published studies conducted by Coalson et al., (1972) and Mahan et al., (1980) with less than 20 kg BW pigs were deemed appropriate to allow the determination of a requirement estimate (NRC, 2012). They date over 30 years from the NRC publication date, emphasizing the lack of research within this BW range pigs and the need for more empirical data to validate the requirement estimate. In Exp. 1, we observed that feeding 0.34 to at least 0.54% STTD P improved G:F and ADG, respectively, with the requirement for maximum ADG being greater than that for maximum G:F. However, diminishing returns were observed in growth rate at STTD P greater than 0.43%. Moreover, at this point of diminishing returns in response to increased STTD P, the grams of STTD P intake per day and grams of STTD P intake per kilogram of gain were 3.52 g/d and 6.31 g/kg of gain. These values are greater than NRC (2012) requirement estimate of 2.99 g/d and the 5.11 g/kg of gain calculated from the 585 g/d of BW gain suggested by NRC (2012) publication. According to Van Milgen and Noblet (1999), approximately 53 to 60% of the energy intake above maintenance of pigs weighing 20 kg goes to protein deposition. Thus, a greater portion of the growth rate of pigs in the current study is likely protein growth, with greater P intake needed to support the protein gain as the muscle tissue contains higher amounts of P compared to fat tissue (Nielsen, 1973). Moreover, the demand for P increases as the ratio of lean tissue growth increases (Jongbloed, 1987). Therefore, the higher requirement for P intake in grams per kilogram of gain observed in the current study may be a consequence of genetic improvement in growth performance and carcass lean meat content of pigs (Partenen et al., 2010).

A considerable amount of phosphorus titration studies of growing-finishing pigs has been reported in the literature (Ekpe et al., 2002, Partanen et al., 2010, Zhai and Adeola, 2013, Adeola et al., 2015). Observations from our study corroborate these studies, which suggest the P

requirements for pig growth performance are greater than the NRC (2012) estimates. As an example, the NRC (2012) STTD P estimate for 25- to 50-kg pigs is 0.31%. Ekpe et al., (2002) estimated the STTD P requirement for 23.5- to 60-kg pigs between 0.35 and 0.38% to support maximum growth rate and feed efficiency. Breakpoints from non-linear broken-line regression models were determined for 19- to 40-kg pigs (Adeola et al., 2015). The STTD P requirement was estimated at 0.39 and 0.41% to maximize ADG and G:F, respectively. These studies, however, evaluated the digestible P requirement of heavier BW pigs compared to the current study.

Concentration of P in the body is closely related to the concentration of Ca, and an excess or deficiency of one mineral may affect the utilization of the other (Crenshaw et al.,2001). Thus, it is important to consider an appropriate ratio between Ca and P for diet formulations. Two different approaches are commonly used in studies that are designed to determine the requirement of Ca or P. They can be structured to contain graded values of the mineral of interest while maintaining the other constant, or alternatively, they can be structured to contain a constant Ca:P ratio. In a study designed to determine the P requirement with a constant level of Ca, a high Ca or wide Ca:P ratio could be detrimental to performance in the low P diets, while the Ca could be a limiting nutrient in high P diets. In low P diets, excess Ca could lead to the formation of Ca-P complexes in the gastrointestinal tract, reducing P digestibility and absorption (Stein et al., 2011; González-Vega and Stein, 2014). In high P diets, Lagos et al. (2019) reported that growth rate was reduced in diets containing low Ca compared to diets containing Ca above the requirement. The authors raised the possibility that binding of Ca may also occur by excess P. According to results from González-Vega et al. (2016), increasing the concentration of STTD Ca in diets containing a constant concentration of STTD P is detrimental to pig performance.

Conversely, the authors also observed that lower STTD Ca concentration in the diet fed during a short period of time was not detrimental to pig growth performance. The current study utilized the approach of maintaining a constant analyzed Ca:P ratio of 1.17:1. Thus, diets with low P concentration were also formulated with lower Ca concentration, which could have potentially favored the low P treatments.

Moreover, Ca release by phytase was not accounted in the diet formulation in Exp. 2. We acknowledge that we formulated the diets based on a constant analyzed Ca:P ratio. Coefficients for STTD of P in feed ingredients were obtained from NRC (2012), and values for STTD of Ca were obtained from Stein et al. (2016). The STTD Ca concentrations in the diets in both experiments were recalculated, including the 0.096% STTD Ca release by phytase as recommended by the manufacturer (DSM Nutritional Products, Inc., Parsippany, NJ). The STTD Ca:STTD P ratio ranged between 1.53:1 to 1.20:1, with wider ratios observed at lower P levels. Therefore, the reduced growth performance in the low P treatments could be further decreased than what would be observed merely due to P inadequacy. Lagos et al. (2019) observed that when the STTD P is provided at the recommended level by NRC (2012) of 0.33%, the ratio to maximize ADG of 11 to 22 kg pigs was at 1.39:1 STTD Ca:STTD P. Detrimental effects with STTD P in excess of NRC (2012) were only observed at STTD Ca levels greater than 0.60%, which was the case of the highest dietary treatments in the current study. The authors also described that the ratio to maximize growth rate when STTD P was provided at 0.42% was 1.28:1 STTD Ca:STTD P. In the current study, the STTD P to optimize growth rate was determined at approximately 0.43%. At this STTD P concentration, the calculated STTD Ca:STTD P ratios were 1.31:1 and 1.28:1 in Exp. 1 and Exp. 2, respectively, corroborating with the results described by Lagos et al. (2019).

In addition, it is worthwhile to consider that approximately 60 to 80% of P in feedstuffs of plant origin is stored in phytic acid, typically in the form of phytate (Eeckout and Da Paepe, 1994). Pigs lack sufficient endogenous phytase to effectively cleave the phosphates from the phytate. Thus, phytate is known as an antinutritional factor in swine diets (Swick and Ivey, 1992) as it reduces P digestibility. A practical and economical solution to this problem consists of adding an exogenous phytase source to swine diets, which has the ability to dephosphorylate the phytate in a step-wise manner and liberate P. As a consequence, P availability to the pig is increased while a need for the addition of expensive inorganic sources of P in the diet is decreased (Selle and Ravindran, 2008).

According to Almeida and Stein (2004), swine diets formulated with the addition of phytase and less inorganic P result in a reduction in P excretion in the environment without negatively effecting growth performance. Wu et al., (2018) also titrated the STTD P in diets for early nursery pigs containing 2,000 FYT of phytase. When phytase was added in the diets, the estimated maximum ADG occurred at 138% of the NRC (2012) using the QP model, while the maximum G:F was estimated at 147 and 116% of the NRC (2012) using the QP and BLL models, respectively. These results are in accordance with the observations in Exp. 2, in which a greater STTD P requirement compared to the NRC (2012) was estimated for late nursery pigs fed diets containing 1,000 FYT phytase. Moreover, compared to Exp. 1, in Exp. 2 more replicates per treatment were utilized and a breakpoint for maximum ADG was estimated through a BLL model at 0.40% STTD P. The estimated breakpoint is fairly consistent with the point of diminishing returns in growth rate in Exp. 1 at 0.43%, suggesting that the recommended manufacturer releasing ability of 1,000 FYT phytase of 0.132% STTD P used in the present study was accurate. In addition, the breakpoints for STTD P intake in grams per day for pigs fed

diets containing phytase were 2.92 and 3.02 g/d. These values are very similar to the NRC (2012) STTD P requirement estimate of 2.99 g/d, suggesting that the NRC requirement estimates of STTD P by nursery pigs are accurate on a grams per day basis. Similar to Exp. 1, in Exp. 2 the STTD P intake in grams per kilogram of gain was greater than the 5.11 g/kg gain calculated from the 585 g/d of BW gain suggested by NRC (2012) publication.

These data provide empirical evidence that the NRC (2012) accurately determines the STTD P requirement by nursery pigs on a grams per day basis. However, as a percentage of the diet, NRC (2012) underestimates the STTD P requirement for G:F and ADG of 11- to 23-kg nursery pigs. Our results suggest that, depending on the response criteria and statistical model, the STTD P level as a percentage of the diet to optimize growth performance of 11- to 23-kg pigs fed diets without or with 1,000 FYT added phytase ranged from 0.34 to 0.42% STTD P. Practical implications of this are that many swine nutritionists use the dietary percentages as a baseline for setting requirement estimates which can lead to under estimating STTD P concentrations. However, if accurate feed intake measurements are available, the NRC estimated requirements on a grams per day basis can be translated into more accurate baseline dietary percentage recommendations. Also, this supports that the underlying assumptions used in developing NRC requirement estimates are accurate.

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Table 1.1 Diet composition, Exp. 1 and 2 (as-fed basis)[1]

Item	Exp. 1		Exp. 2	
	0.26% STTD P[2]	0.53% STTD P	0.30% STTD P	0.58% STTD P
Ingredients, %				
Corn	64.95	63.10	65.79	63.73
Soybean meal, 46,5% CP	31.72	31.85	31.66	31.80
Monocalcium phosphate, 21% P	0.52	1.92	0.00	1.60
Limestone	1.00	1.30	0.74	1.05
Sodium chloride	0.60	0.60	0.65	0.65
L-Lysine HCl	0.48	0.48	0.48	0.48
DL-Methionine	0.21	0.21	0.21	0.21
L-Threonine	0.16	0.16	0.16	0.16
L-Tryptophan	0.03	0.03	0.03	0.03
L-Valine	0.08	0.08	0.08	0.08
Phytase[3]	---	---	0.04	0.04
Vitamin premix[4]	0.13	0.13	---	---
Trace mineral premix[5]	0.10	0.10	---	---
Vitamin and trace mineral premix[6]	---	---	0.15	0.15
Copper chloride[7]	0.04	0.04	0.04	0.04
Total	100	100	100	100
Calculated analysis				
Standardized ileal digestible amino acids, %				
Lysine	1.33	1.33	1.33	1.33
Isoleucine:lysine	57	56	57	57
Leucine:lysine	117	116	117	116
Metthionine:lysine	37	37	37	37
Methionine and cysteine:lysine	58	58	58	58
Threonine:lysine	60	60	60	60
Tryptophan:lysine	19	19	19.1	19.1
Valine:lysine	67	67	67	67
Net energy, kcal/kg	2,429	2,385	2,452	2,401
Crude protein, %	21.3	21.2	20.5	20.4
Calcium, %	0.59	0.94	0.47	0.84
Phosphorus, %	0.51	0.80	0.40	0.71
Standardized total tract digestible phosphorus, %	0.26	0.53	0.30	0.58
Available phosphorus, %	0.19	0.49	0.23	0.54
Calcium:phosphorus	1.17	1.18	1.17	1.17

[1]In Exp. 1, diets were fed from 11.4- to 22.8-kg BW. Diets were blended to form the intermediate treatments: 0.30, 0.33, 0.38, 0.43, and 0.48 40% STTD P. In Exp. 2, diets were

fed from 11.1- to 22.5- kg BW. Diets were blended to form the intermediate treatments: 0.33, 0.38, 0.43, 0.48, and 0.53% STTD P.

[2]STTD P = standardized total tract digestible phosphorus.

[3]Ronozyme HiPhos 2500 (DSM Nutritional Products, Parsippany, NJ) provided 1,000 FYT per kg of feed, releasing an assumed 0.15% avP and 0.132% STTD P.

[4]Provided per kg of premix: 8,818,490 IU vitamin A; 1,102,311 IU vitamin D; 35,273 IU vitamin E; 3,527.4 mg vitamin K; 30.9 mg vitamin B12; 39,683 mg niacin; 22,046 mg pantothenic acid; 6,614 mg riboflavin.

[5]Provided per kg of premix: 165 g Zn from Zn sulfate; 165 g Fe from iron sulfate; 40 g Mn from manganese oxide; 17 g Cu from copper sulfate; 0.3 g I from calcium iodate; 0.3 g Se from sodium selenite.

[6]Provided per kg of premix: 5,346,210 IU vitamin A; 1,338,206 IU vitamin D; 100,211 IU vitamin E; 1,671.1 mg vitamin K; 21.4 mg vitamin B12; 29,061 mg niacin; 15,366 mg pantothenic acid; 4,008 mg riboflavin, 73.5 g Zn from Zn sulfate; 66.8 g Fe from iron sulfate; 26.7 g Mn from manganese oxide; 10 g Cu from copper sulfate; 0.5 g I from calcium iodate; 0.2 g Se from sodium selenite.

[7]Supplemental copper provided in the form of tri-basic copper chloride (TBCC; Intellibond C; Micronutrients, Indianapolis, IN) at 150 ppm.

Table 1.2 Chemical analysis of diets (as-fed basis; Exp. 1)[1,2]

	STTD P[3], %						
Item, %	0.26	0.30	0.33	0.38	0.43	0.48	0.53
Dry matter	87.78	87.75	88.18	87.84	87.87	88.02	88.14
Crude protein	19.6	20.2	21.4	21.5	21.4	20.3	20.7
Ether extract	2.4	2.4	2.3	2.3	2.3	2.4	2.3
Ash	3.81	4.25	4.56	4.98	4.88	4.93	5.14
Calcium	0.65	0.74	0.73	0.85	0.90	0.82	0.88
Phosphorus	0.44	0.49	0.54	0.64	0.66	0.71	0.75

[1]A representative sample of each diet was collected from 6 feeders, homogenized, then analyses were conducted on composite samples (Ward Laboratories, Inc., Kearney, NE).

[2]Low (0.26% STTD P) and high (0.53% STTD P) diets were blended at the farm by a robotic feeding system to create the 0.30, 0.33, 0.38, 0.43, and 0.48% STTD P dietary treatments.

[3]STTD P = standardized total tract digestible phosphorus.

Table 1.3 Chemical analysis of diets (as-fed basis; Exp. 2)[1,2]

	STTD P[3], %						
Item, %	0.30	0.33	0.38	0.43	0.48	0.53	0.58
Dry matter	88.42	88.53	89.20	88.83	88.90	88.51	88.60
Crude protein	18.23	19.68	20.40	19.60	20.60	19.20	19.23
Ether extract	2.30	2.03	2.05	2.23	2.33	2.15	2.13
Ash	3.62	3.96	4.52	4.57	4.55	4.76	5.11
Calcium	0.54	0.61	0.65	0.79	0.77	0.83	0.95
Phosphorus	0.33	0.42	0.46	0.52	0.58	0.60	0.66

[1]A representative sample of each diet was collected from 6 feeders, homogenized, then analyses were conducted on composite samples (Ward Laboratories, Inc., Kearney, NE).

[2]Low (0.26% STTD P) and high (0.53% STTD P) diets were blended at the farm by a robotic feeding system to create the 0.30, 0.33, 0.38, 0.43, and 0.48% STTD P dietary treatments.

[3]STTD P = standardized total tract digestible phosphorus.

Table 1.4 Least square means for growth performance of nursery pigs fed increasing standardized total tract digestible (STTD) P from 11- to 23-kg body weight (BW), Exp. 1[1,2]

	STTD P[3], %									
	0.26	0.30	0.33	0.38	0.43	0.48	0.53		Probability, *P* =	
Item[4] % of NRC[5]	80	90	100	115	130	145	160	SEM	Linear	Quadratic
d 0 to 21										
ADG, g	513	510	533	532	566	563	573	11.6	<0.001	0.718
ADFI, g	782	764	776	780	818	824	828	19.4	0.004	0.603
G:F, g/kg	656	667	687	682	692	684	693	7.4	<0.001	0.066
STTD P, g/d	2.03	2.29	2.56	2.97	3.52	3.95	4.39	0.082	0.001	0.418
STTD P, g/kg gain	3.85	4.41	4.89	5.53	6.31	7.19	7.68	0.067	0.001	0.579
BW, kg										
d 0	11.4	11.4	11.4	11.4	11.4	11.4	11.4	0.29	0.935	0.933
d 21	22.2	22.2	22.6	22.7	23.3	23.3	23.5	0.92	0.001	0.759

[1] A total of 1,080 barrows and gilts (PIC; 337 × Camborough, initial pen average BW of 11.4 ± 0.29 kg) were used in a 21-d growth trial with 23 to 27 pigs per pen and 6 pens per treatment. Two groups of pigs were weaned at approximately 21 d of age, fed a common phase 1 and phase 2 diet for 21 or 24 d post-weaning, then fed experimental diets.

[2] Low (0.26% STTD P) and high (0.53% STTD P) diets were blended at the farm by a robotic feeding system to create the 0.30, 0.33, 0.38, 0.43, and 0.48% STTD P dietary treatments.

[3] STTD P= Standardized total tract digestible phoshphorus.

[4] ADG= average daily gain. ADFI= average daily feed intake. G:F= gain-to-feed ratio. BW= body weight.

[5] The NRC requirement estimate for nursery pigs from 25 to 55 lb, expressed as a percentage of the diet, is 0.33% STTD P. Therefore, treatment concentrations represented 80, 90, 100, 115, 130, 145, and 160% of the NRC (2012) requirement.

Table 1.5 Least square means for growth performance of nursery pigs fed increasing standardized total tract digestible (STTD) P from 11- to 23-kg body weight (BW), Exp. 2[1,2]

	STTD P[3], %[4]									
	0.30	0.33	0.38	0.43	0.48	0.53	0.58		Probability, *P* =	
Item[5] % of NRC[6]	90	100	115	130	145	160	175	SEM	Linear	Quadratic
d 0 to 21										
ADG, g	515	523	539	549	547	542	545	8.6	<0.001	0.009
ADFI, g	747	749	753	768	773	770	762	15.4	0.060	0.198
G:F, g/kg	691	700	716	715	708	706	716	5.6	0.002	0.027
STTD P, g/d	2.24	2.47	2.86	3.30	3.71	4.07	4.42	0.072	<0.001	0.321
STTD P, g/kg gain	4.34	4.72	5.31	6.01	6.79	7.51	8.10	0.049	<0.001	0.223
BW, kg										
d 0	11.1	11.1	11.1	11.1	11.1	11.1	11.1	0.24	0.978	0.990
d 21	22.0	22.2	22.5	22.7	22.6	22.6	22.6	0.39	0.028	0.125

[1] A total of 2,140 pigs (PIC 337 × Camborough, initial pen average BW of 11.1 ± 0.24 kg) were used in a 21-d growth trial with 24 to 27 pigs per pen and 12 pens per treatment. Pigs were weaned at approximately 19 d of age, fed a common phase 1 and phase 2 diets for 25 d post-weaning, then fed experimental diets.

[2] Low (0.30% STTD P) and high (0.58% STTD P) diets were blended at the farm by a robotic feeding system to create the 0.33, 0.38, 0.43, 0.48, and 0.53% STTD P dietary treatments.

[3] STTD P = Standardized total tract digestible phoshphorus.

[4] Phytase (Ronozyme HiPhos, DSM Nutritional Products, Parsippany, NJ) was included at 1000 FYT/kg releasing an assumed 0.15% avP and 0.132% STTD P.

[5] ADG= average daily gain. ADFI= average daily feed intake. G:F= gain-to-feed ratio. BW= body weight.

[6] The NRC requirement estimate for nursery pigs from 25 to 55 lb, expressed as a percentage of the diet, is 0.33% STTD P. Therefore, treatment concentrations represented 90, 100, 115, 130, 145, 160, and 175% of the NRC (2012) requirement.

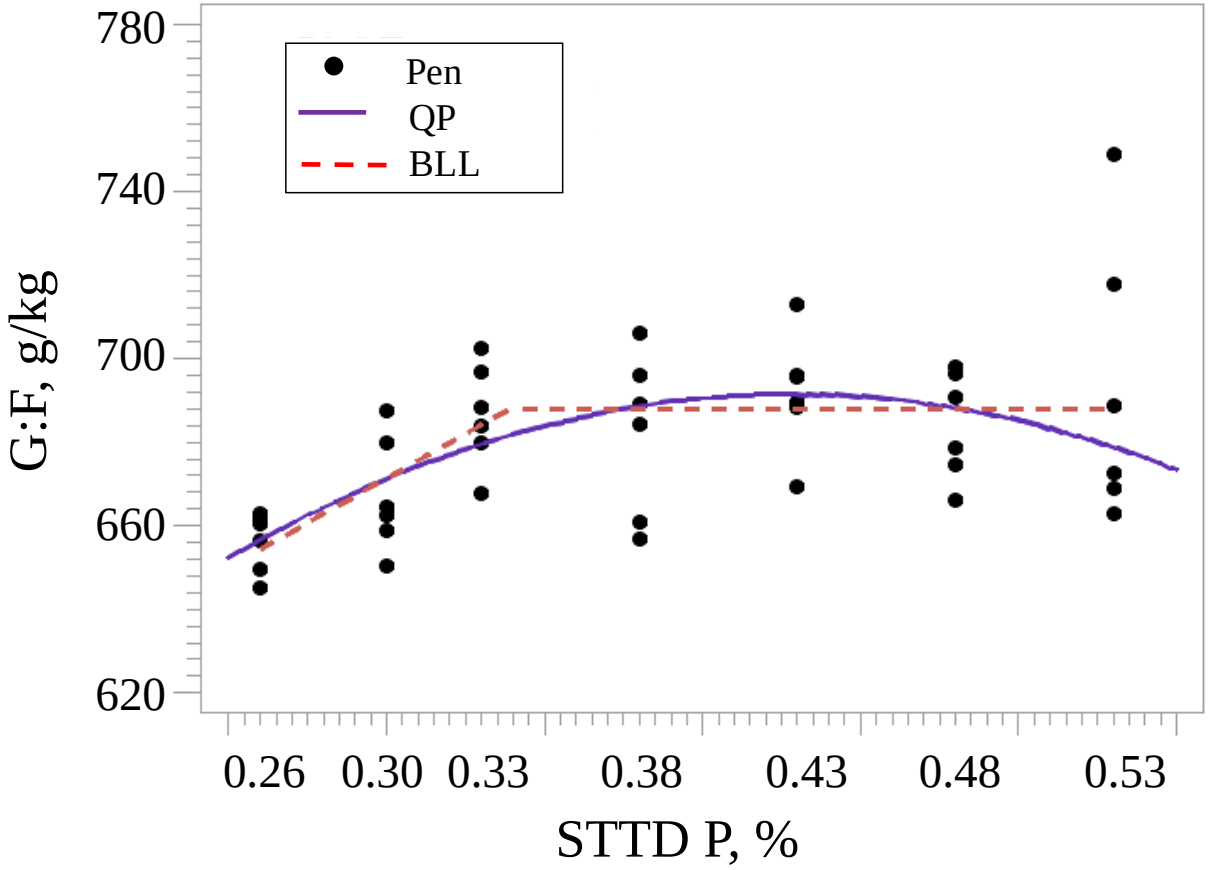

Figure 1.1 Fitted quadratic polynomial (QP) and broken-line linear (BLL) regression models on feed efficiency (G:F) as a function of increasing standardized total tract digestible (STTD) P in 11- to 23-kg pigs in Exp. 1. The QP model estimated the maximum mean G:F at 0.42% (95% CI: [0.36, >0.53]%), with 99% of maximum G:F achieved at 0.36%. The estimated regression equation was G:F, g/kg = 456.59 + 1107.49 × (STTD P) – 1307.16 × (STTD P)^2. The BLL breakpoint was estimated at 0.34% (95% CI: [0.30, 0.37]%).The estimated regression equation was G:F, g/kg = 696.63 – 427.26 × (0.3358 – STTD P) if STTD P < 0.34%, and G:F, g/kg = 696.63 if STTD P ≥ 0.34%.

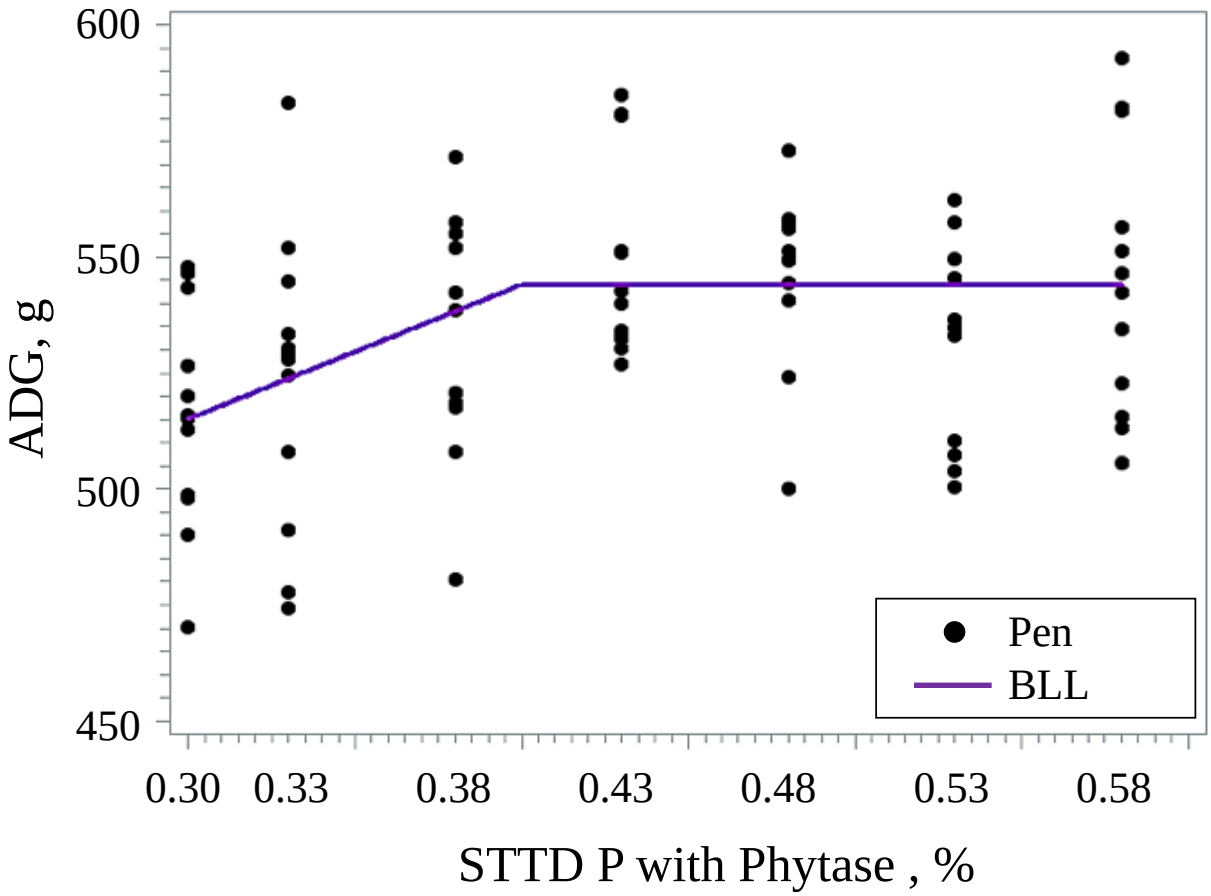

Figure 1.2 Fitted broken-line linear (BLL) regression model on average daily gain (ADG) as a function of increasing standardized total tract digestible (STTD) P with 1,000 added phytase units in 11- to 23-kg pigs in Exp 2. The BLL breakpoint was estimated at 0.40% (95% CI: [0.33, 0.47]%). Based on the best fitting model, the estimated regression equation was ADG, g = 543.97 – 289.79 × (0.3993 – STTD P) if STTD P <0.40%, and ADG, g = 543.97 if STTD P ≥ 0.40%.

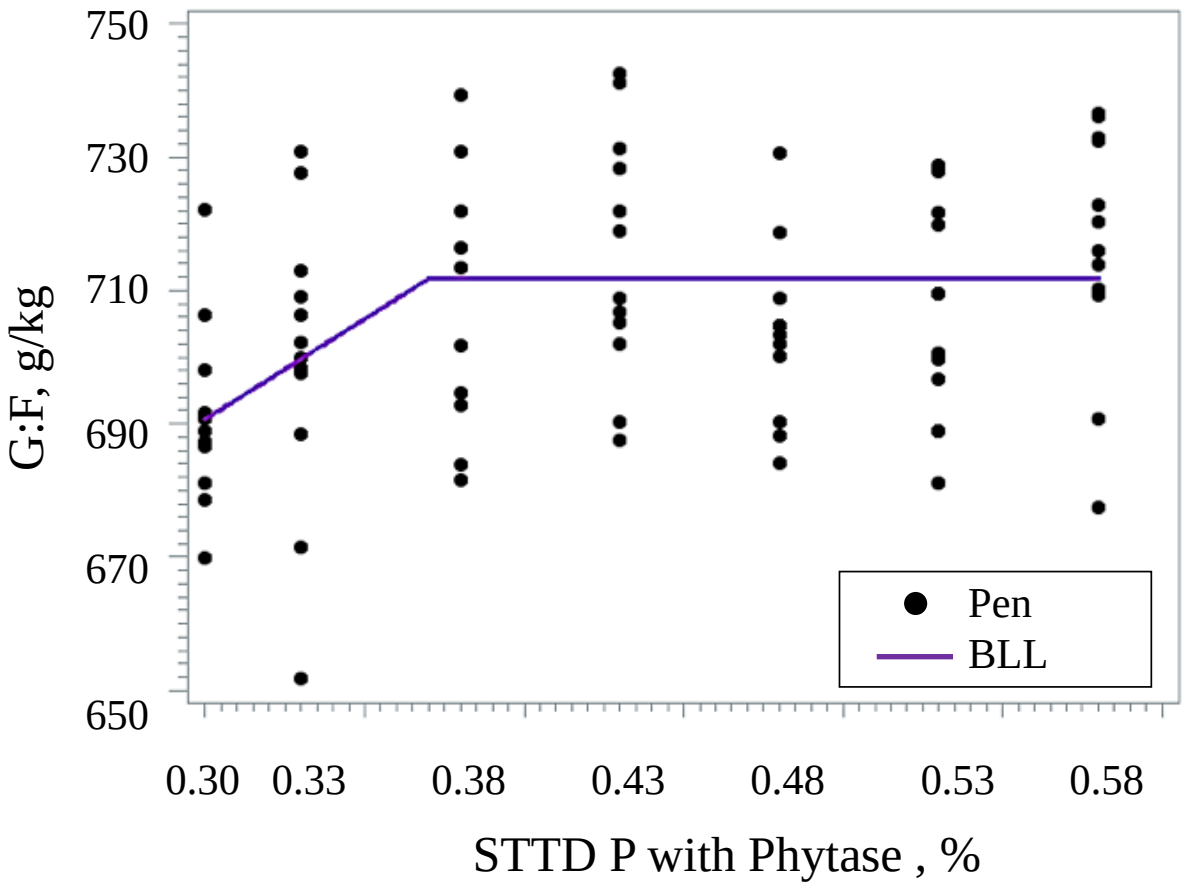

Figure 1.3 Fitted broken-line linear (BLL) regression model on feed efficiency (G:F) as a function of increasing standardized total tract digestible (STTD) P with 1,000 added phytase units in 11- to 23-kg pigs in Exp. 2. The BLL breakpoint was estimated at 0.37% (95% CI: [0.29, 0.45]%). Based on the best fitting model, the estimated regression equation was G:F, g/kg = 711.76 – 301.08 × (0.37 – STTD P) if STTD P <0.37%, and G:F, g/kg = 711.76 if STTD P ≥0.37%.

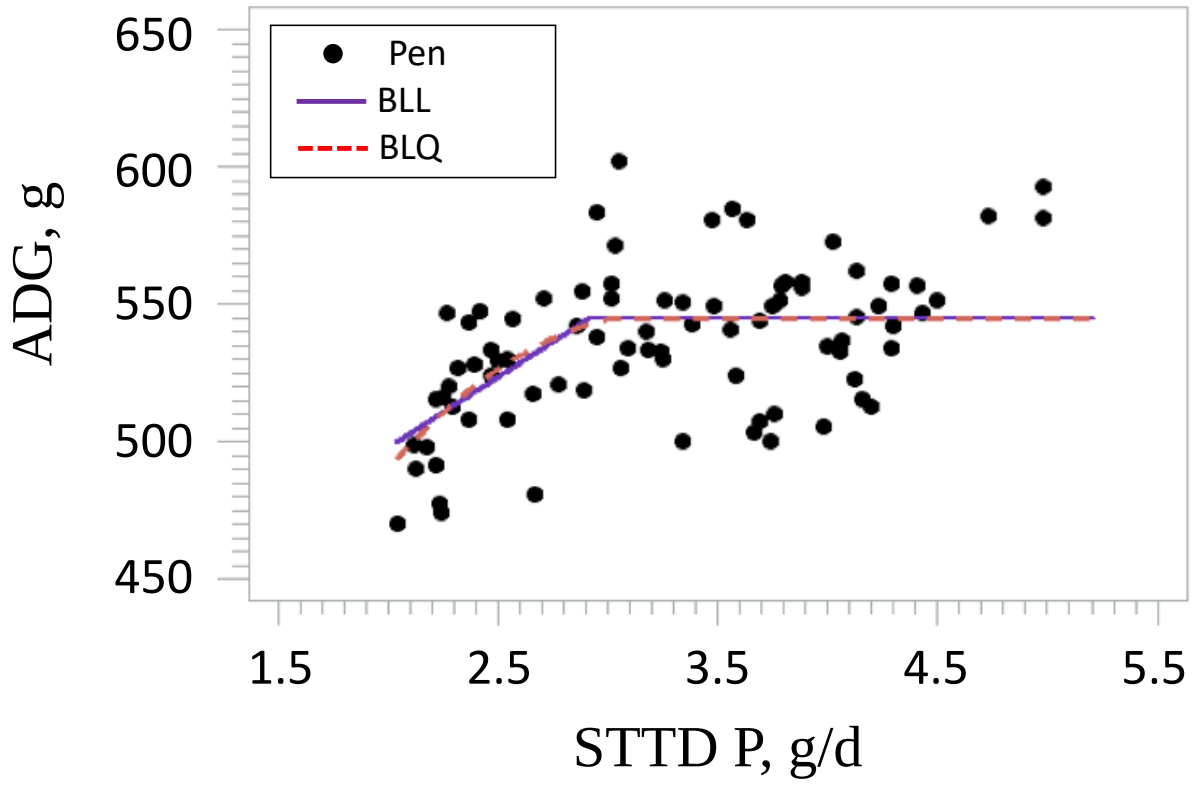

Figure 1.4 Fitted broken-line linear (BLL) and broken-line quadratic (BLQ) regression models on average daily gain (ADG) as a function of increasing standardized total tract digestible (STTD) P intake in grams per day in 11- to 23-kg pigs fed diets with 1,000 added phytase units in Exp 2. The BLL breakpoint was estimated at 2.92 g/d (95% CI: [2.56, 3.27g/d]) STTD P. Based on the BLL model, the estimated regression equation was ADG, g = 545.11 – 51.3991 × (2.917 – STTD P in g/d) if STTD P intake < 2.92 g/d, and ADG, g = 545.11 if STTD P intake ≥ 2.92 g/d. The BLQ breakpoint was estimated at 3.02 g/d (95% CI: [3.00, 3.03g/d]) STTD P. Based on the BLQ model, the estimated regression equation was ADG, g = 544.96 – 17.2077 × (3.019 – STTD P in g/d) – 35.7972 × (3.019 – STTD P in g/d)^2 if STTD P intake < 3.02 g/d,, and ADG, g = 544.96 if STTD P intake ≥ 3.02 g/d.

Chapter 2 - Standardized total tract digestible phosphorus requirement of 24- to 130-kg pigs

ABSTRACT

A study was conducted to determine the standardized total tract digestible phosphorus (STTD P) requirement for 24- to 130-kg finishing pigs housed under commercial conditions. A total of 1,130 barrows and gilts (PIC 359 × 1050, Hendersonville, TN; initially 24.2 kg) were used, with 26 to 27 pigs per pen with 7 replicates per treatment. Pens of pigs were allotted to treatments in a randomized complete block design with body weight (BW) as the blocking factor. The dietary treatments were fed in 4 phases and were formulated to contain 80, 90, 100, 115, 130, and 150% of the NRC (2012) requirement estimate for finishing pigs within each phase. Weight ranges for each phase were: 27 to 49, 49 to 76, 76 to 90, and 90 to 130 kg. Treatments were achieved by increasing the amount of monocalcium phosphate at the expense of corn in the diet with no added phytase. All diets were formulated to contain a similar 1.14:1 to 1.16:1 total Ca:P ratio across treatments in all phases. Increasing STTD P resulted in a quadratic response ($P < 0.05$) in average daily gain (ADG), gain-to-feed ratio (G:F), and final BW. The greatest improvement was observed with STTD P at 130% of NRC for ADG and final BW and at 115% STTD P for G:F. Average daily feed intake increased linearly (linear, $P < 0.05$) with the inclusion of STTD P. Increasing STTD P resulted in an increase (quadratic, $P < 0.05$) in hot carcass weight (HCW) and carcass ADG, with the greatest response observed with STTD P at 130% of NRC. There was a marginally significant response (quadratic, $P < 0.10$) in carcass G:F, with the greatest improvement with STTD P at 115% of NRC. Carcass yield decreased (linear, $P < 0.05$) with increasing STTD P, while there was a marginally significant (linear, $P < 0.10$) decrease in backfat and increase in fat-free lean. At the end of the study, a metacarpal was

collected and analyzed for bone ash. Increasing STTD P resulted in an increase (linear, $P < 0.05$) in bone ash weight and percentage ash. For ADG and G:F, the quadratic model demonstrated the best fit. The maximum response in ADG and G:F was estimated at 122% and 116% of NRC STTD P, respectively. The broken-line linear model best fit the data for percentage bone ash, with a plateau achieved at 131% of the NRC STTD P. In conclusion, the estimated STTD P requirement of 24- to 130-kg ranged from 116% to 131% of the NRC publication (2012) requirement estimate.

Key words: bone mineralization, finishing pigs, growth, modeling, phosphorus

INTRODUCTION

Phosphorus (**P**) is an inorganic element that is essential for growth performance and development and maintenance of the skeletal system (Berndt and Kumar, 2009; NRC, 2012). Approximately two-thirds of the body concentration of P is found in the pig skeleton, while the remaining P is found in muscle tissues where it is involved in different biological functions (Crenshaw, 2001). Diets formulated with excess P can lead to an increase P excretion, negatively effecting the environment. In addition, this mineral is the third most expensive component in swine diets after energy and protein (Fan et al., 2001). Thus, diets are typically formulated to avoid excess P, with low margins of safety.

In 2012, the NRC started to report the requirements for P in a standardized total tract digestibility (**STTD**) basis, which are based on a factorial approach. The NRC (2012) emphasized a need for empirical data to validate the model-derived digestible P requirement. Moreover, the concentration of P is greater in muscle tissue compared to adipose tissue (Nielsen, 1973). This implies that improvements in the genetic potential for lean growth in pigs result in a greater P requirement. The highest final pig body weight (**BW**) from the empirical estimates

reported in the NRC (2012) model was approximately 109 kg, it dates 8 years before the NRC publication, and this is the only data point past 70 kg BW. Today, pigs are marketed at heavier BW, and the genetic selection for high protein deposition warrants a revaluation of the digestible P requirement.

Furthermore, current statistical capabilities for modeling dose-response studies has allowed for a more precise estimation of the concentration of P needed to optimize different response criteria. Therefore, the objective of this study was to determine the effects of STTD P on growth performance, carcass characteristics, and bone mineralization of 24- to 130-kg pigs housed under commercial conditions.

MATERIALS AND METHODS

The Kansas State University Institutional Animal Care and Use Committee (Manhattan, KS) approved all experimental procedures in this study.

Animals and Diets

The study was conducted at a commercial research-finishing site in southwestern Minnesota (New Horizon Farms, Pipestone, MN). The facility was naturally ventilated and double-curtain-sided. One barn was used containing 42 pens (3.05 × 5.49 m^2) with completely slatted concrete flooring and a deep pit for manure storage. Each pen was equipped with a 4-hole stainless steel, dry self-feeder (Thorp Equipment, Thorp, WI) and 1 cup waterer. The facility was equipped with a computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN) capable of measuring and recording daily feed additions to individual pens. Thirteen barrows and fourteen gilts (PIC 359 × 1050, Genus PIC, Hendersonville, TN) were housed in each pen and were allowed ad libitum access to feed and water throughout the experiment.

A total of 1,130 pigs (initial average BW of 24.2 kg) were used in a 111-d growth trial. After placement in the finishing facility, pigs were fed a common diet until the initiation of the trial. The common diet was formulated to be at the pigs' STTD P requirement based on NRC (2012) estimates (0.33% STTD P). On d 0 of the trial, pens of pigs were sorted by average BW and randomly allotted to 1 of 6 dietary treatments in a randomized complete block design with BW as the blocking factor. There were 7 replicate pens per treatment with 26 or 27 pigs per pen.

All treatment diets were manufactured at the New Horizon Farms Feed Mill in Pipestone, MN and fed in meal form. The experimental diets were corn-soybean meal–based and were fed in 4 different phases (Table 1). The diets were formulated to contain 80, 90, 100, 115, 130, and 150% of the NRC (2012) publication requirement for finishing pigs within each phase. The NRC (2012) requirement for phases 1 (25- to 50-kg), 2 (50- to 75-kg), 3 (75- to 100-kg), and 4 (100- to 135-kg), expressed as a percentage of the diet, are estimated as 0.31, 0.27, 0.24, and 0.21% STTD P, respectively. Phase 1 diets were fed from d 0 to 29 (24.1- to 49.1-kg); phase 2 diets were fed from d 29 to 56 (49.1- to 75.5-kg); phase 3 diets were fed from d 56 to 70 (75.5- to 89.7-kg); and phase 4 diets were fed from d 70 to 111 (89.7- to 130.4-kg). The STTD P concentrations were achieved by increasing the amount of monocalcium phosphate at the expense of corn. There was no added phytase. Diets were formulated to an expected similar total Ca:P ratio of 1.14:1 to 1.16:1 across dietary treatments in all phases with the inclusion of limestone at the expense of corn.

Pens of pigs were weighed, and feed disappearance was recorded on d 0, 29, 56, 70, 99, and 111 to determine average daily gain (**ADG**), average daily feed intake (**ADFI**), gain-to-feed ratio (**G:F**), grams of STTD P intake per day, and grams of STTD P intake per kilogram of gain. The STTD P, based on formulated values, were multiplied by ADFI to calculate grams of STTD

P intake per day. The total grams of STTD P intake, based on formulated values, were divided by total BW gain to calculate the grams of STTD P intake per kilogram of gain.

Carcass and Bone Data Collection

On d 99, the 2 heaviest pigs in each pen were selected, weighed, and sold according to standard farm procedures. These pigs were used in calculation of pen growth performance, but not carcass characteristics. On d 111, final pen weights were taken and one barrow and one gilt with intermediate weights were selected and tattooed with a pen identification. These pigs were transported to a commercial abattoir in northwest Iowa (Natural Food Holdings Inc., Sioux Center, IA) for processing and collection of metacarpal bones. Following processing, the left front feet were separated at the junction of carpals and radius and ulna, and individually placed in a zip-lock plastic bag with a permanent identification tag within the bag. These feet were transferred on dry ice to the Kansas State University Swine Laboratory and stored at -20℃ until analysis of bone mineral content.

The remaining pigs were individually tattooed with the specific pen identity on the shoulder to allow for carcass measurements to be recorded on a pen basis. These pigs were transported to a commercial packing plant in southwestern Minnesota (JBS Swift and Company, Worthington, MN) for processing and carcass data collection. Carcass measurements included hot carcass weight (**HCW**), loin depth, backfat depth, and percentage lean. Fat depth and loin depth were measured with an optical probe inserted between the third and fourth last rib (counting from the ham end of the carcass) at a distance approximately 7 cm from the dorsal midline. Percentage carcass yield was calculated by dividing the average pen HCW collected at the plant by the average final live weight at the farm before transport. Carcass ADG was

calculated by multiplying the overall ADG by percentage carcass yield. Carcass G:F was calculated by dividing the overall ADFI by carcass ADG.

Bone Ash Analysis

After thawing overnight, the feet were autoclaved for 1 h at 121℃. The third and fourth metacarpals of each foot were removed. These bones were cleaned of extraneous soft tissue, and refrozen. The third metacarpal was dried at ambient temperature for 24 h and cut in half and weighed. They were wrapped in cheesecloth to keep their tag ID and defatted by petroleum ether using a Soxhlet apparatus for 7 d. De-fated metacarpals were placed in a 105℃-drying oven for 24 h to determine the dry fat-free weight. Bones were then ashed in a muffle furnace at 600℃ for another 24 h to determine percentage ash. Ash is expressed as a percentage of dried fat-free bone weight.

Chemical Analysis

Representative diet samples were obtained from 6 feeders of each treatment approximately 3 d after the beginning and 3 d before the end of the phase and delivered to the Kansas State University Swine Laboratory, Manhattan, KS, and stored at -20°C until analysis. Samples of the diets were combined within dietary treatment, and a composite sample from each treatment was analyzed in duplicate (Ward Laboratories, Inc., Kearney, NE; Table 2). Samples were analyzed for Ca and P (method 985.01; AOAC International, 1990).

Statistical Analysis

Experimental growth data were analyzed as a randomized complete block design with block as a random effect and pen as the experimental unit. The study was structured as a split-plot design in a randomized complete block design for the bone data. The whole-plot treatments included the different STTD P concentrations. Within each of the dietary treatments, there was a

one-way treatment structure with gender as the factor level. A random effect of block by treatment was used to identify the pair of pigs (one barrow and one gilts) within each pen as the experimental unit for gender. The two-way interaction between dietary treatments and gender was tested, and no significant interactions were observed. Response variables were analyzed using generalized linear and non-linear mixed models. Polynomial contrasts were implemented to evaluate the functional form of the dose response to increasing dietary STTD P on ADG, ADFI, G:F, BW, grams of STTD P intake per day, grams of STTD P intake per kilogram of gain, HCW, carcass ADG, carcass G:F, carcass yield, carcass backfat, carcass fat-free lean, and carcass loin depth. Backfat depth, loin depth, and percentage lean were adjusted to a common HCW. The Kenward–Roger method was used to adjust the denominator degrees of freedom and correct the standard errors for bias (Littell, et al., 2006). The coefficients for the unequally spaced linear and quadratic contrasts were derived using the IML procedure in SAS (Version 9.3, SAS Institute Inc., Cary, NC). Statistical models were fit using GLIMMIX procedure of SAS. Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

In addition, the effects of the STTD P levels on overall ADG, G:F, and percentage bone ash were fit using procedures described by Gonçalves et al. (2016) . Models were expanded to account for heterogeneous residual variances when needed. Competing statistical models included a linear (**LM**), quadratic polynomial (**QP**), broken-line linear (**BLL**), and broken-line quadratic (**BLQ**). Dose response models were compared based on the Bayesian information criterion (**BIC**), where the smaller the value, the better (Milliken and Johnson, 2009). A decrease in BIC greater than 2 was considered a significant improvement in model fit. The 95% confidence interval of the estimated requirement to reach maximum performance or to reach

plateau performance was computed. Results reported correspond to inferences yielded by the best fitting models.

RESULTS

Chemical Analysis

The average values of analyzed P across dietary phases were approximately 20, 10, 6, 5, 5, and 5% lower than formulated values for treatments that represented 80, 90, 100, 115, 130, and 150% of NRC estimates across phases, respectively (Table 2). These values are still within the acceptable analytical variation based on the AAFCO's sample program (AAFCO, 2015) with the exception of the lowest P treatment. Although variation in analyzed P existed, analyzed P content still increased linearly with increasing STTD P treatments. The average values of analyzed Ca across dietary phases were approximately 22, 18, 27, 27, 32, and 15% greater than formulated values for treatments that represented 80, 90, 100, 115, 130, and 150% of NRC estimates across phases, respectively. Average values of analyzed Ca was approximately 23% higher than formulated values. Chemical analysis of dietary Ca is typically more variable, with a higher coefficient of variation than P (Wu et al., 2018). According to the AAFCO's sample program, the acceptable variability in the laboratory analyses of Ca are approximately 30% given the formulated Ca levels used in this study (AAFCO, 2015). According to the analyzed values of Ca and P, the analyzed Ca:P ratio in the final diets were greater than formulated, with the average across phases varying from 1.40:1 to 1.74:1 among treatments.

Growth Performance

From d 0 to 56 (phases 1 and 2), which corresponded to the grower period, increasing the STTD P increased ADG (quadratic, $P < 0.05$; Table 3) driven by an increase in ADFI (quadratic, $P < 0.05$). The greatest improvement occurred as the STTD P increased from 80 to 115% of the

NRC requirement estimate, starting to decrease at the highest STTD P concentration of 150%. Feed efficiency was not affected by dietary treatment ($P > 0.10$). From d 56 to 111 (phases 3 and 4), which corresponded to the finisher period, increasing STTD P increased ADG (quadratic, $P < 0.05$) driven by an improvement in G:F (quadratic, $P < 0.05$). The greatest improvement in G:F occurred as the STTD P increased from 80 to 115% of the NRC requirement estimate and started to decrease at higher levels. For ADG, the greatest increase was observed from 80 to 130% of the NRC requirement estimate and then it decreased at the highest STTD P. During this period, feed intake was not affected ($P > 0.10$) by dietary treatment. The grams of STTD P intake per day increased in a quadratic fashion ($P < 0.05$) during the grower period, and in a linear manner during the finisher period ($P < 0.05$). The grams of STTD P intake per kilogram of gain increased linearly ($P < 0.05$) for the grower and finisher periods, with a marginal quadratic response during the finisher period ($P < 0.10$).

For the overall study, increasing STTD P increased ADG and final BW (quadratic, $P < 0.05$). The greatest increase in ADG and final BW was observed as STTD P increased from 80 to 130% of NRC requirement estimates, with both ADG and final BW decreasing at the highest STTD P concentration of 150% of NRC estimates. Similarly, feed efficiency improved (quadratic, $P < 0.05$) as STTD P increased from 80 to 115% of the NRC requirement and started to worsen thereafter. Average daily feed intake increased (linear, $P < 0.05$) as STTD P increased, however, with the greatest feed intake observed at 130% STTD P of the NRC requirement estimate.

Homogeneous variance was used for ADG and heterogeneous variance was used for G:F models. For ADG (Figure 1), the best fitting model was the QP. Based on the best fitting model, the STTD P concentration for maximum ADG was estimated at 122% (95% CI: [104, 143%]) of

the NRC (2012) requirement estimates within phases. The estimated QP regression equation was:

ADG, g = 651.36 + 531.33 × (STTD P as % of NRC) – 216.90 × (STTD P as % of NRC)^2.

Similarly, the best fitting model for G:F (Figure 2) was the QP. The STTD P concentration for maximum G:F was estimated at 116% (95% CI: [90, >150%]) of the NRC (2012) requirement for each phase. Based on this model, the estimated regression equation was:

G:F, g/kg = 338.34 + 108.98 × (STTD P as % of NRC) – 46.7864 × (STTD P as % of NRC)^2.

Carcass Characteristics

For carcass characteristics, HCW increased (quadratic, $P < 0.05$) as STTD P increased up to 130% of the NRC (2012) requirement estimate, and then started to decrease at the higher STTD P concentration. Similarly, carcass ADG increased (quadratic, $P < 0.05$) with the greatest response observed with STTD P at 130% of the NRC (2012) requirement estimate. There was also a marginally significant response (quadratic, $P < 0.10$) in carcass G:F, with the greatest improvement observed as STTD P increased from 80 to 115% of the NRC requirement estimate, starting to worsen at higher STTD P levels. Carcass yield decreased (linear, $P < 0.05$) with increasing STTD P, while there was a marginally significant linear decrease ($P < 0.10$) in backfat and increase in fat-free lean. No statistically significant difference ($P > 0.10$) was observed for loin depth.

Bone Mineralization

For bone characteristics, increasing STTD P resulted in an improvement (linear, $P < 0.05$) in fat-free bone ash weight (Table 4). However, there was no evidence for difference ($P >$

0.10) in fat-free bone ash weight due to gender when the model was adjusted to account for differences in HCW between barrows and gilts. Similarly, ash as a percentage of fat-free dried bone increased (linear, $P < 0.05$) as STTD P increased, with diminishing returns in percentage bone ash at STTD P concentration greater than 130% of NRC estimates. In addition, barrows had significantly greater ($P < 0.05$) percentage bone ash than gilts.

Homogeneous variance was used to model the bone mineralization data. The model that best fit the percentage bone ash (Figure 3) was the BLL. The STTD P concentration for maximum percentage bone ash was estimated at 131% (95% CI: [113, 148%]) of the NRC (2012) requirement estimate for each phase. The estimated regression equation was:

Bone ash, % = 62.10 – 2.54 × (1.31 – STTD P as % of NRC) if STTD P as % of NRC < 1.31%,

Bone ash, % = 62.10 if STTD P as % of NRC ≥ 1.31%

DISCUSSION

Phosphorus requirement estimates for pigs reported in the latest NRC (2012) publication are expressed on a standardized total tract digestible basis. According to Almeida and Stein (2010), values for STTD P of feed ingredients are additive in mixed diets and can be used to formulate diets for pigs without compromising performance. However, that P requirements reported by the NRC are based on factorial estimates. Few empirical studies were considered appropriate to be included in the NRC model, and only three of them had an average pig BW greater than 60 kg, which dated from 8 to 31 years before the NRC publication (Thomas and Kornegay, 1981, Hastad et al., 2004). It is well known that muscle tissue constitutes the second most abundant P reserve in the body after skeletal tissue, with minimum P found in the adipose tissue (Nielsen, 1973). Pig genotype can influence the extent of bone and muscle tissue

deposition, which can lead to different dietary P requirements (Hittmeier et al., 2006, Alexander et al., 2008). In today's pig production, pigs are not only marketed at heavier weights but are also highly selected for high lean tissue deposition. The current study was designed to provide more information on the STTD P requirement of modern genotype growing-finishing pigs and to validate NRC model requirement estimates.

Ekpe et al., (2002) conducted a study to estimate the STTD P requirement for 25- to 50-kg pigs. The authors have observed that the requirement to optimize growth rate and feed efficiency is approximately 113 to 127% of the NRC (2012) requirement estimates. Adeola et al., (2015) estimated the phosphorus requirement for 19- to 40-kg pigs through broken-line regression models. The breakpoints estimated in this study occurred at approximately 126 to 132% of the NRC (2012) estimates to maximize ADG and G:F. Quadratic improvements in ADG due to increasing available P concentrations were also reported in studies conducted by Stahly et al. (2001) and Arouca et al. (2012) with 9- to 119-kg and 95- to 120-kg pigs. The authors also reported quadratic improvements in feed efficiency of late finishing pigs.

The majority of the aforementioned studies were considered short period studies compared to the current experiment, which evaluated the P requirement over the entire growing and finishing periods. Observations from our study have determined the requirements to optimize growth rate and feed efficiency at 116 to 122% of the NRC estimates, corroborating results from the earlier studies. Moreover, low dietary P concentrations can negatively affect ADG and G:F (Reinhart and Mahan, 1986), which is in agreement with the quadratic improvements in growth performance observed in the study herein. Conversely, Nieto et al. (2016) evaluated the effects of varying STTD P levels from 0.186 to 0.336% on performance of 48- to 80-kg pigs. The authors reported that ADG, final BW, and G:F were not affected by

increasing digestible P. Similarly, O'Quinn et al., (1997) and Hastad et al., (2004), when evaluating the effects of increasing available P levels on performance of 25- to 118-kg and 88- to 109-kg, respectively, observed no effects of dietary P levels on ADG. Moreover, a quadratic effect of available P on feed efficiency was observed by O'Quinn et al., (1997), while no evidence of dietary P treatment effect on feed efficiency was observed in the study conducted by Hastad et al., (2004).

In the current study, the diets were formulated to maintain a similar Ca:P ratio across treatments. However, analyzed values of total Ca were higher and total P were lower than formulated values. This resulted in an average Ca:P ratio of 1.74:1 in the lowest STTD P treatment, and an average of 1.40:1 in the highest STTD P treatment across phases. Previous studies have demonstrated that a wide Ca:P ratio can be detrimental to pig performance, which is more evident when diets are marginal or low in P (Reinhart and Mahan, 1986, Wu et al., 2018). According to González-Vega et al. (2016), growth performance of 25- to 50-kg pigs was reduced with increasing concentrations of STTD Ca, especially when diets contained low concentrations of P. Similarly, Merriman et al. (2017) have shown that diets containing a total Ca:P ratio greater than 1.18:1 resulted in reduced growth performance of 100- to 130-kg pigs fed diets at or below the P estimated requirement by NRC (2012). In the current study, the reduced growth performance observed in the low P treatments could be a result of a wide Ca:P ratio instead of purely due to P inadequacy. Therefore, this could lead to an overestimation of the P requirement.

During the grower period, the requirement of STTD P in grams of digestible P intake to support optimum growth rate was between 6.6 to 7.4 g/d. During the finisher period, the requirement of STTD P in grams of digestible P intake to support optimum growth rate was between 7.1 to 8.3 g/d. When published data are recalculated, with the exception of the study by

Nieto et al. (2016), the highest digestible P intake in grams per day observed for 25- to 50-kg and 34- to 56-kg pigs in the studies by O'Quinn et al. (1997) and Hastad et al. (2004) were lower (6.7 and 5.9 g/d, respectively) than the values observed in our study. Similarly, the highest intake of digestible P in grams per day for 80- to 118- kg and 88- to 109-kg pigs in the studies by O'Quinn et al. (1997) and Hastad et al. (2004) were lower (6.9 and 6.1 g/d, respectively) than the values observed in our study. This may help explain the discrepancies in responses for ADG between the current study and those stated above. Furthermore, using ADG as response criteria, the digestible P intakes in grams per day observed herein are greater than NRC (2012) estimates (4.59 to 5.78 and 6.11 to 5.95 g/d during the grower and finisher periods, respectively). As the ratio of lean tissue growth increases, more P is required by the pig to support this growth (Jongbloed, 1987). Therefore, results from our study may reflect the changes over time in swine genetics and the improvements made in performance and lean tissue growth of pigs (Partanen et al., 2010).

In our study, pigs fed diets with increasing levels of STTD P showed an increase in feed intake, whereas Hastad et al. (2004), Arouca et al. (2012), and Nieto et al. (2016) observed no effects of dietary P concentration on ADFI. Phosphorus may be involved in appetite control (Ruan et al., 2007), and a P-deficiency could result in a reduction in feed intake by growing-finishing pigs (Aubel et al., 1936, Sørensen et al., 2018). However, it is worth noting that the feed intake responses in the current study could also have been due to an energy response as the diets were not balanced by energy across treatments. Although, the dietary energy differences would not be expected to impact feed intake to the extent reported. Even though there was an energy dilution with increasing the STTD P concentrations, the caloric intake increased with

increasing the P levels. This suggests that the energy concentration does not entirely explain the feed intake observed in this study.

Considerable research regarding the effects of increasing dietary P has observed no evidence of P concentration impacting carcass backfat thickness, muscle depth, and lean meat quantity and percentage (O'Quinn et al., 1997, Arouca et al., 2012, Nieto et al., 2016). These results are in agreement with the current study, in which no evidence of STTD P effect on loin depth was observed. However, our study demonstrated a marginal quadratic decrease in backfat and increase in fat-free lean as the digestible P concentration increased up to 130% of NRC estimates. It is hypothesized that when the P concentration is below the pig requirement, which was approximately 122% of NRC for growth rate in this study, the rate of muscle accretion is reduced (Bertram, 1995). According to Bertram (1995), when P is below the requirement, pigs also have a higher proportion of fat deposition in the carcass, which could explain the results observed in our study. Cromwell et al. (1970) also reported that pigs from 18- to 93-kg fed diets with the lowest P concentration had the greatest backfat thickness. A short study from 95- to 120-kg reported no effect of P concentration in the diet on carcass yield (Arouca et al., 2012). Similarly, results from a study conducted by O'Quinn et al. (1997) with 25- to 118- kg pigs have shown that carcass yield was not influenced by dietary P levels. Our study, however, demonstrated a linear decrease in percentage carcass yield with increasing dietary STTD P levels. The reason for the reduction in yield observed in our study is not well understood, but this reduction is consistent to observations from other studies with 26- to 127- kg pigs that evaluated different Ca to P ratios in diets adequate in STTD P (Vier et al., 2018a,b).

Calcium and P have specific requirements for bone mineralization (Létourneau-Montminy et al., 2012). Specifically, approximately 96 to 99% of Ca and 70 to 80% of P are

found in the pig skeleton in the form of hydroxyapatite (Crenshaw, 2001). The remainder of the whole-body P is found in soft tissues, where both minerals have little or no common function. Thus, it is necessary to consider the ratio between Ca and P in diet formulation. Our study was structured to maintain a constant Ca:P ratio of 1.14:1 to 1.16:1 across dietary treatments. It has previously been reported that a wide Ca:P ratio is detrimental in diets with low P (González-Vega et al., 2016). The authors reported that approximately 1.05:1 to 1.61 Ca:P ratio is required to obtain optimum amount of bone ash in 25- to 50-kg pigs in diets with low to excess P (González-Vega et al., 2016). In our study, the analyzed Ca:P ratios in the final diets were greater than the calculated and varied from 1.40:1 to 1.74:1 among treatments. Thus, a wide Ca:P ratio would have the potential to impair bone mineralization in pigs fed diets with low P concentration. However, bone ash of heavier pigs from 100- to 130-kg pigs linearly increased as the dietary concentration of Ca increased regardless of the concentration of P (Merriman et al., 2017). Therefore, the analyzed Ca:P ratio observed in final diets would not be expected to negatively impact bone mineralization in the current study.

Bone mineralization results from our trial determined the requirement for maximum bone ash as a percentage of dry, fat-free metacarpal at 131% of NRC estimates, as opposed to 116 to 122% requirements for growth performance. These results are in agreement with the findings reported by Mahan et al. (1982), Hastad et al. (2004), Partanen et al. (2010), and Saraiva et al. (2012), in which the P requirement to maximize bone mineralization is greater than the level required to optimize growth performance. According to Crenshaw (2001), the deposition of P in bones continues after the deposition of P required for maximum muscle growth. We observed a greater percentage bone ash for barrows compared to gilts, but the magnitude of this difference is relatively small. Results from other research have reported no evidence for differences in bone

ash content between sexes (Crenshaw et al., 1981, Teixeira et al., 2016), with only a tendency for boars to have lower percentage bone ash compared to gilts or barrows (Crenshaw et al., 1981).

Our study evaluated the bone mineralization as a percentage of dry, fat-free weight of metacarpals. It is known that the amount of water and fat in the bone can vary according to the age of the animal, type of bone, and nutritional status (Crenshaw et al., 2001). Thus, bone mineral content should be expressed as a percentage of the dry, fat-free weight (Crenshaw, 2001). The selection of bone type for the assessment of whole-body mineral content is more critical in young pigs than in older pigs (Crenshaw et al., 2009). The femur provided a better fit to dietary P inputs in 25- to 30-kg pigs compared to the fibula. However, in 40- to 120-kg pigs, no differences in femur, front feet and hind feet were observed when evaluating such bones as predictors of whole-body mineral content (Crenshaw et al., 2009).

Standardized total tract digestible P requirement estimates of growing and finishing pigs in the current study were determined using different response criteria, including growth performance, carcass characteristics, and bone mineralization. Together, results from our study indicate that, as a percentage of the diet, NRC (2012) underestimates the STTD P requirement for growing-finishing pigs. Our results suggest that, depending on the response criteria, the STTD P level to optimize growth performance, carcass characteristics, and bone mineralization of 24- to 130-kg pigs ranged from 116 to 131% of NRC (2012) dietary percentage estimates across all phases.

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Table 2.1 Diet composition for Phases 1 to 4 diets (as-fed basis)[1]

Item	Phase 1	Phase 2	Phase 3	Phase 4
Ingredient, %				
Corn	66.71 – 65.25	77.45 – 76.17	84.24 – 83.09	84.91 – 83.93
Soybean meal, 46.5% CP	30.78 – 30.88	20.15 – 20.24	13.58 – 13.66	13.13 – 13.20
Limestone	0.95 – 1.18	0.90 – 1.10	0.85 – 1.03	0.83 – 0.98
Monocalcium phosphate, 21% P	0.45 – 1.59	0.43 – 1.42	0.38 – 1.27	0.26 – 1.03
Sodium chloride	0.35	0.35	0.35	0.35
L-Lysine HCl	0.35	0.35	0.30	0.25
DL-Methionine	0.12	0.07	0.02	0.01
L-Threonine	0.11	0.12	0.10	0.08
L-Tryptophan	0.01	0.02	0.02	0.01
Vitamin premix[2]	0.08	0.08	0.08	0.08
Trace mineral premix[3]	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Standardized ileal digestible amino acids, %				
Lysine	1.21	0.95	0.75	0.70
Isoleucine:lysine	61	59	60	64
Leucine:lysine	127	136	151	161
Methionine:lysine	33	32	30	31
Methionine and cysteine:lysine	56	57	58	60
Threonine:lysine	61	63	65	67
Tryptophan:lysine	18.7	18.2	18.2	18.5
Valine:lysine	66	67	70	74
Total lysine, %	1.35	1.07	0.85	0.80
Net energy, kcal/kg	2,445 – 2,407	2,509 – 2,476	2,549 – 2,520	2,555 – 2,529
Crude protein, %	20.7	16.5	13.8	13.6
Calcium, %	0.56 – 0.84	0.50 – 0.75	0.45 – 0.67	0.42 – 0.61
Phosphorus, %	0.49 – 0.73	0.44 – 0.65	0.40 – 0.59	0.37 – 0.53
STTD P[4], %	0.25 – 0.46	0.22 – 0.40	0.19 – 0.36	0.17 – 0.31
Available phosphorus, %	0.17 – 0.42	0.15 – 0.37	0.13 – 0.32	0.11 – 0.27
Calcium:phosphorus	1.15	1.15	1.14	1.15

[1] Treatment were formulated to contain 80, 90, 100, 115, 130, and 150% of NRC (2012) STTD P estimates across dietary phases (0.31, 0.31, 0.24, 0.21% for phases 1, 2, 3, and 4, respectively). Phase 1 diets were fed from d 0 to 29 (24.1- to 49.1-kg), phase 2 from d 29 to 56 (49.1- to 75.5-kg), phase 3 from d 56 to 70 (75.5- to 89.7-kg), and phase 4 from d 70 to 111 (89.7- to 130.4-kg).

[2] Provided per kg of premix: 8,818,490 IU vitamin A; 1,102,311 IU vitamin D; 35,273 IU vitamin E; 3,527.4 mg vitamin K; 30.9 mg vitamin B12; 39,683 mg niacin; 22,046 mg pantothenic acid; 6,614 mg riboflavin.

[3] Provided per kg of premix: 165 g Zn from Zn sulfate; 165 g Fe from iron sulfate; 40 g Mn from manganese oxide; 17 g Cu from copper sulfate; 0.3 g I from calcium iodate; 0.3 g Se from sodium selenite.

[4] Standardized total tract digestible phosphorus.

Table 2.2 Chemical analysis of experimental diets (as-fed-basis)[1]

	STTD P, % of NRC (2012)[2,3,4,5]					
Item	80	90	100	115	130	150
Total calcium (Ca), %						
Phase 1	0.74	0.74	0.76	0.79	0.73	0.91
Phase 2	0.51	0.55	0.59	0.74	0.91	0.84
Phase 3	0.58	0.54	0.57	0.59	1.11	0.84
Phase 4	0.53	0.58	0.85	0.89	0.67	0.69
Total phosphorus (P), %						
Phase 1	0.40	0.43	0.47	0.49	0.56	0.62
Phase 2	0.31	0.43	0.48	0.49	0.57	0.57
Phase 3	0.33	0.36	0.36	0.44	0.46	0.57
Phase 4	0.31	0.39	0.48	0.53	0.54	0.58

[1] Representative samples of treatment diets were taken from 6 feeders per dietary treatment 3 d after the beginning and 3 d before the end of the phase and stored at -20°C. After blending, subsamples were submitted to Ward Laboratories, Inc. (Kearney, NE) for analyses.

[2] Phase 1 calculated STTD P were 0.25, 0.28, 0.31, 0.36, 0.40, and 0.46%, and calculated total P were 0.49, 0.52, 0.56, 0.61, 0.66, and 0.73% for 80, 90, 100, 115, 130, and 150% of the NRC (2012) estimate, respectively.

[3] Phase 2 calculated STTD P were 0.22, 0.24, 0.27, 0.31, 0.35, and 0.40% and calculated total P were 0.44, 0.46, 0.49, 0.54, 0.59, and 0.65% for 80, 90, 100, 115, 130, and 150% of the NRC (2012) estimate, respectively.

[4] Phase 3 calculated STTD P were 0.19, 0.22, 0.24, 0.28, 0.31, and 0.36%, and calculated total P were 0.40, 0.42, 0.45, 0.49, 0.53, and 0.59 % for 80, 90, 100, 115, 130, and 150% of the NRC (2012) estimate, respectively.

[5] Phase 4 calculated STTD P were 0.17, 0.19, 0.21, 0.24, 0.27, and 0.31% and calculated total P were 0.37, 0.39, 0.42, 0.45, 0.49, and 0.53% for 80, 90, 100, 115, 130, and 150% of the NRC (2012) estimate, respectively.

Table 2.3 Least square means for growth performance and carcass characteristics of growing-finishing pigs fed increasing standardized total tract digestible (STTD) P from 24- to 130-kg body weight (BW)[1]

	STTD P, % of NRC (2012) requirement[2]							Probability, *P*=	
Item	80	90	100	115	130	150	SEM	Linear	Quadratic
Grower period (d 0 to 56)									
ADG, g	886	910	904	933	930	917	11.7	0.001	0.003
ADFI, g	1,874	1,922	1,919	1,984	1,983	1,930	35.5	0.017	0.004
G:F, g/kg	473	474	471	470	469	476	4.9	0.958	0.271
STTD P intake, g/d	4.3	5.0	5.5	6.6	7.4	8.3	0.12	<0.001	0.009
STTD P intake, g/kg gain	4.9	5.4	6.1	7.0	7.9	9.1	0.07	<0.001	0.351
Finisher period (d 56 to 111)									
ADG, g	1,000	1,001	1,010	1,020	1,045	998	12.7	0.208	0.006
ADFI, kg	2,899	2,912	2,910	2,873	2,958	2,926	26.5	0.254	0.792
G:F, g/kg	345	344	347	355	353	341	3.5	0.818	<0.001
STTD P intake, g/d	5.0	5.7	6.5	7.1	8.3	9.5	0.07	<0.001	0.842
STTD P intake, g/kg gain	5.0	5.7	6.4	7.1	7.9	9.4	0.09	<0.001	0.063
Overall period (d 0 to 111)									
ADG, g	941	955	956	975	986	956	6.3	0.001	<0.001
ADFI, g	2,374	2,407	2,401	2,417	2,456	2,415	23.2	0.033	0.102
G:F, g/kg	397	397	398	404	401	396	3.2	0.574	0.002
BW, kg									
d 0	24.2	24.1	24.2	24.2	24.1	24.2	0.73	0.992	0.954
d 56	73.8	75.1	75.0	76.7	77.0	75.6	1.26	<0.001	<0.001
d 111	127.9	129.3	130.1	131.9	133.6	129.7	1.12	<0.001	<0.001
Carcass characteristics[3]									
HCW, kg	93.0	94.5	94.5	95.4	96.5	93.9	0.72	0.012	<0.001
Carcass ADG, g[4]	685	698	694	706	712	692	4.6	0.029	<0.001
Carcass G:F, g/kg[5]	289	290	289	292	290	287	2.4	0.469	0.063
Carcass yield, %	72.8	73.1	72.6	72.3	72.2	72.4	0.24	0.027	0.368
Backfat, mm[6,7]	19.0	18.4	18.3	18.6	18.0	18.1	-	0.073	0.580
Fat-free lean, %[6,7]	55.0	55.4	55.4	55.3	55.6	55.5	-	0.097	0.519
Loin depth, mm[6,7]	64.1	64.7	64.2	64.5	64.7	64.3	-	0.796	0.651

[1] A total of 1,130 pigs (337 × 1050, PIC, initially 24.1 kg BW) were used in a 111-d growth trial with 26 to 27 pigs per pen and 7 pens per treatment.

[2] All treatments contain variable concentrations of STTD P that represent 80, 90, 100, 115, 130, and 150% of the NRC (2012) requirement for pigs within phases.

[3] 877 pigs were transported to a commercial packing plant for processing and data collection (Swift and Company, Worthington, MN).

[4] Carcass average daily gain = overall average daily gain × carcass yield.

[5] Carcass G:F = carcass average daily gain/overall average daily feed intake.

[6] SEM for backfat were 0.370, 0370, 0.376, 0.373, and 0.367, SEM for % lean were 0.247, 0.247, 0.251, 0.244, 0.249, and 0.245, and SEM for loin depth were 6.17, 6.17, 6.29, 6.08, 6.23 and 6.10 for 80,90,100,115,130, and 150% of the NRC (2012) requirement, respectively.

[7] Adjusted for HCW.

Table 2.4 Least square means for bone mineralization of growing-finishing pigs fed increasing standardized total tract digestible (STTD) P from 24- to 130-kg body weight (BW)[1]

									Probability, P =[2]		
	STTD P, % of NRC (2012)[3]						Gender		Treatment		Gender
Item[4]	80	90	100	115	130	150	Barrow	Gilt	Linear	Quadratic	
Ash bone weight, g	8.47	8.75	9.05	9.25	9.56	10.03	9.24	9.13	0.001	0.840	0.501
SEM	0.177	0.178	0.180	0.188	0.179	0.178	0.108	0.115			
Ash, %	60.8	60.7	61.1	61.5	61.9	61.9	61.5	61.2	0.001	0.373	0.036
SEM	0.18	0.19	0.19	0.20	0.19	0.19	0.107	0.116			

[1] A total of 1,130 pigs (337 × 1050, PIC, initially 24.1 kg BW) were used in a 111-d growth trial with 26 to 27 pigs per pen and 7 pens per treatment. At the end of the study, 84 pigs (2 pigs/pen, 1 barrow/1gilt) nearest the mean live weight of the pen were subsampled and shipped to a processing facility for collection of metacarpals for bone mineralization analysis (Natural Foods Holdings, Inc., Sioux Center, IA). The 3rd metacarpals were autoclaved for 1h. After cleaning, bones were placed in Soxhlets containing pretoleum ether for 7 d as a means of removing water and fat. They were then dried at 105^0C for 24 h, and then ashed at 600^0C for 24 h.

[2] The two-way interaction was tested and no evidence for significant interactions were observed for ash bone weight and bone percentage ash.

[3] All treatments contain variable concentrations of STTD P that represent 80, 90, 100, 115, 130, and 150% of the NRC (2012) requirement for pigs within phases.

[4] Adjusted for HCW.

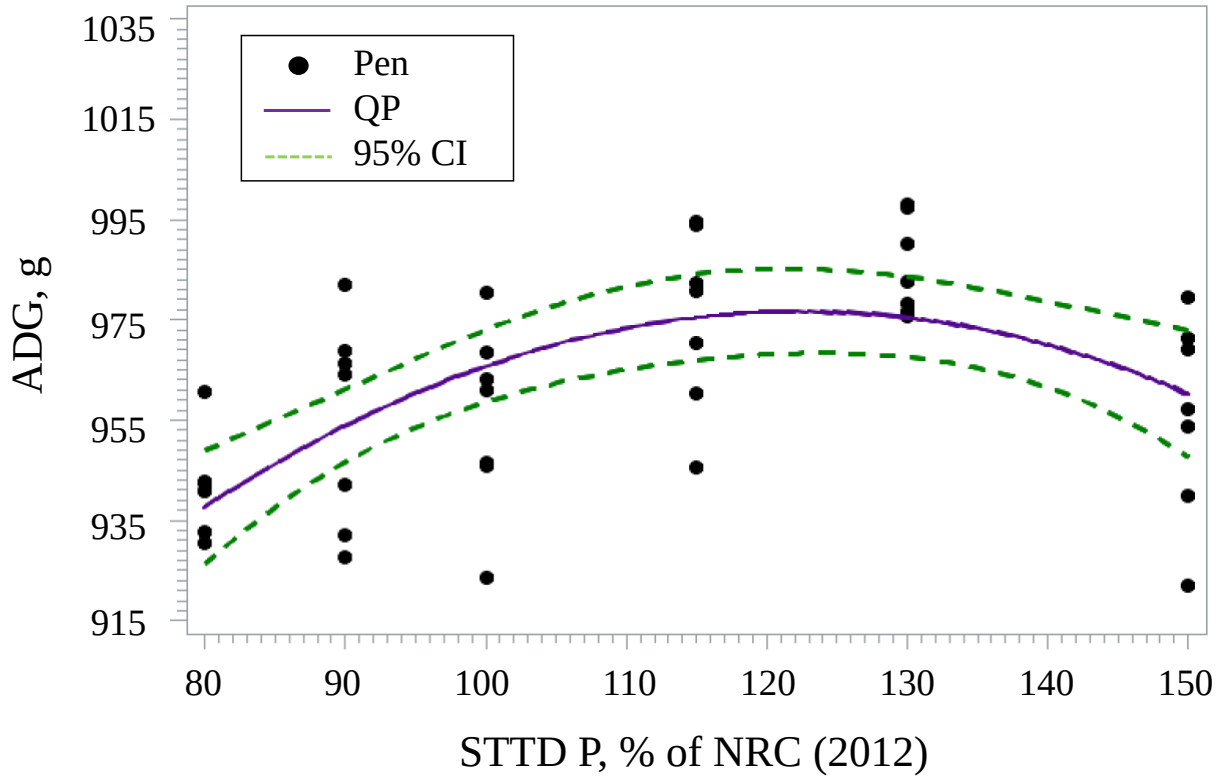

Figure 2.1 Fitted quadratic polynomial (QP) regression model for ADG as a function of increasing standardized total tract digestible (STTD) P in 24- to 130-kg pigs. The QP model estimated the maximum mean ADG at 122% (95% CI: [104, 143%]) of the NRC (2012) recommendations within phases. Based on the best fitting model, the estimated regression equation was ADG, g = 651.36 + 531.33 × (STTD P) – 216.90 × (STTD P)^2.

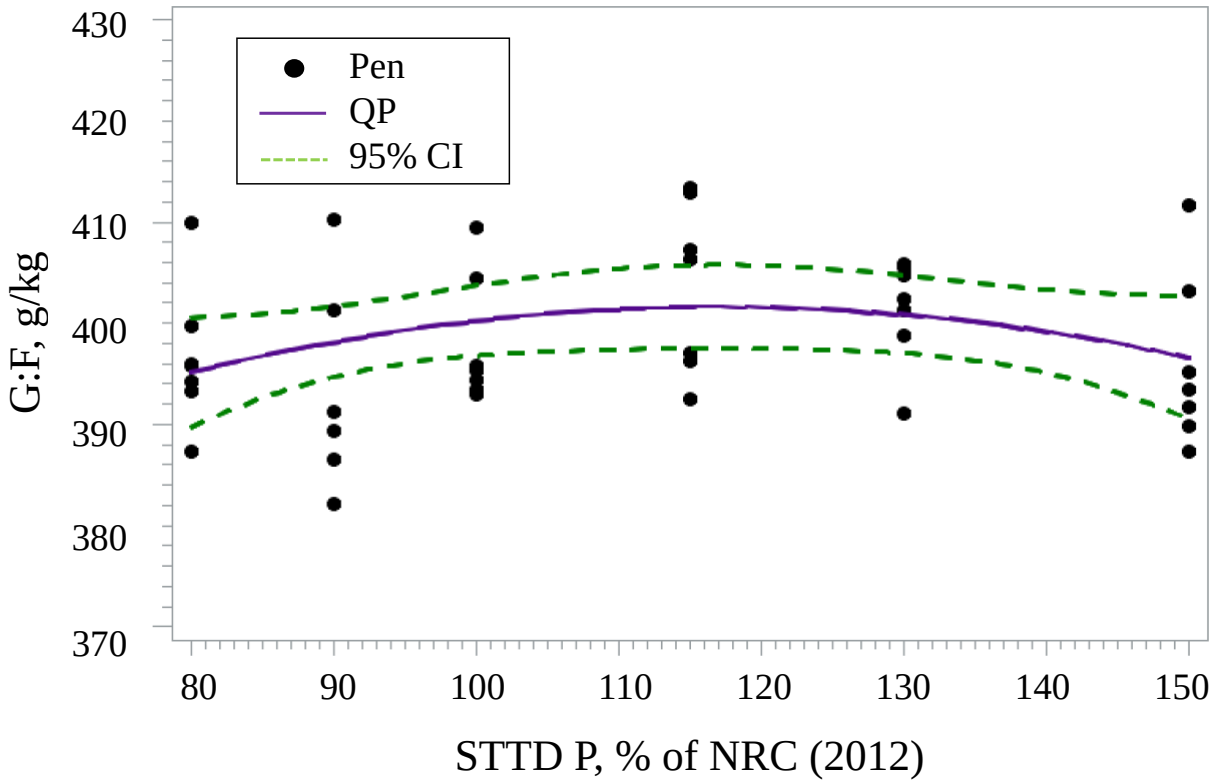

Figure 2.2 Fitted quadratic polynomial (QP) regression model for G:F as a function of increasing standardized total tract digestible (STTD) P in 24- to 130-kg pigs. The QP model estimated the maximum mean G:F at 116% (95% CI: [90, >150%]) of the NRC (2012) recommendations within phases. Based on the QP model, the estimated regression equation was G:F, g/kg = 338.34 + 108.98 × (STTD P) – 46.7864 × (STTD P)^2.

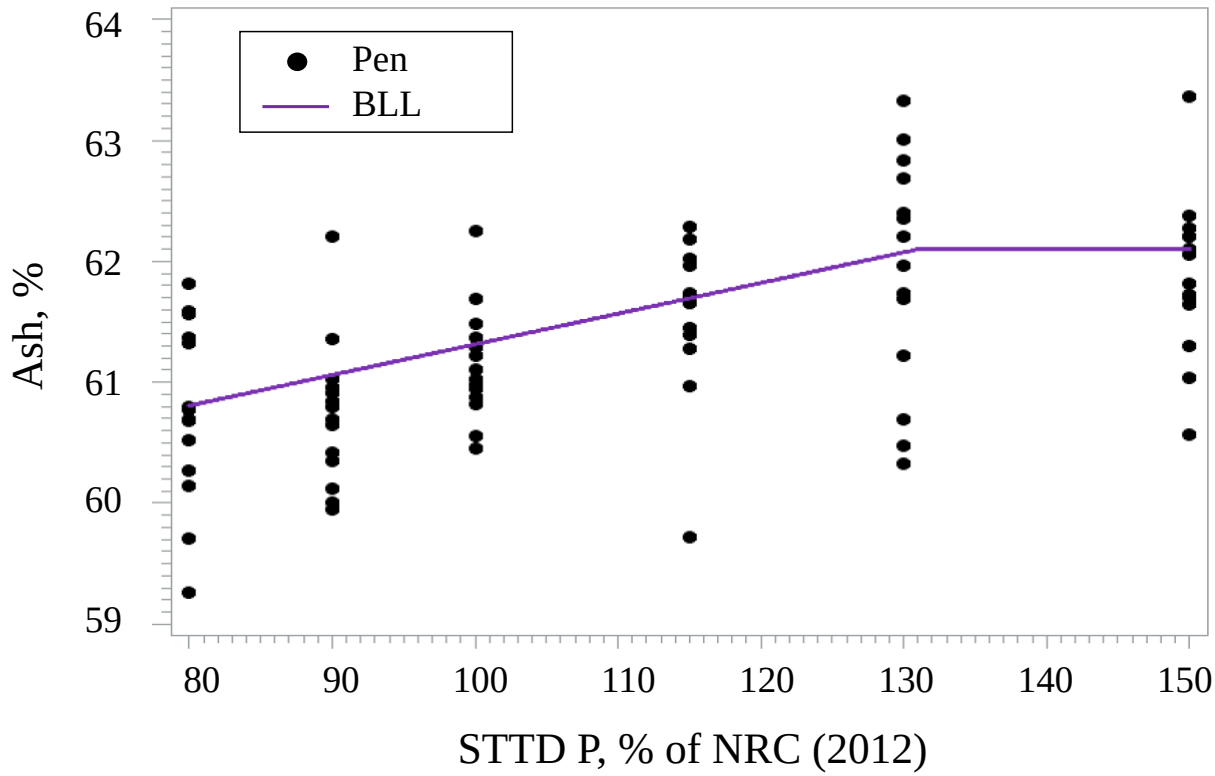

Figure 2.3 Fitted broken line linear (BLL) regression model for bone percentage ash as a function of increasing standardized total tract digestible (STTD) P in 24- to 130-kg pigs. The BLL model estimated the maximum mean percentage ash at 131% (95% CI: [113, 148%]) of the NRC (2012) recommendations within phases.]%). Based on the best fitting model, the estimated regression equation was Bone ash, % = 62.1000 – 2.5374 × (1.31 – STTD P as % of NRC) if STTD P as % of NRC <1.31%, and Bone ash, % = 62.100 if STTD P as % of NRC ≥1.31%.

Chapter 3 - Calcium to phosphorus ratio requirement of 26- to 127-kg pigs fed diets with or without phytase

ABSTRACT

Two experiments were conducted to determine the effects of calcium to phosphorus (**Ca:P**) ratio in diets adequate in standardized total tract digestible (**STTD**) P on performance of 26- to 127-kg pigs fed diets with or without phytase. Pens of pigs (*n*=1,134 in Exp. 1 and *n*=1,215 in Exp. 2, initially 26.3 and 25.3 kg) were blocked by body weight (**BW**) and allotted to treatments in a randomized complete block design. There were 27 pigs per pen with 7 and 9 replicates per treatment in Exp. 1 and 2, respectively. Treatments were formulated to contain 0:75:1, 1.00:1, 1.25:1, 1.50:1, 1.75:1, and 2.00:1 analyzed Ca:P ratios in Exp.1, and 0:75:1, 1.00:1, 1.25:1, 1.50:1, and 2.00:1 analyzed Ca:P ratios in Exp. 2. These correspond to a range of 0.96:1 to 2.67:1 and 0.95:1 to 2.07:1 STTD Ca:STTD P ratios in Exp.1 and 2, respectively. Exp. 2 diets contained 1,000 phytase units of Ronozyme Hiphos 2500 (DSM Nutritional Products, Inc., Parsippany, NJ) with release values of 0.132% STTD P, 0.144% total Ca, and 0.096% STTD Ca. Diets contained 122% of NRC (2012) STTD P estimates for the weight range across 4 phases. In Exp. 1, increasing Ca:P ratio increased (quadratic, $P < 0.05$) average daily gain (**ADG**) and average daily feed intake (**ADFI**). Feed efficiency (**G:F**) worsened (quadratic, $P < 0.05$) at the highest ratio. Hot carcass weight (**HCW**) and bone ash increased (quadratic, $P < 0.05$) while carcass yield decreased (linear, $P < 0.10$) with increasing Ca:P ratio. The maximum responses in ADG, HCW, and bone ash were estimated at 1.38:1, 1.25:1, and 1.93:1 analyzed Ca:P and at 1.82:1, 1.64:1, and 2.57:1 STTD Ca:STTD P, respectively. In Exp. 2, increasing Ca:P ratio increased (quadratic, $P < 0.05$) ADG and bone ash, and improved G:F (linear, $P < 0.05$). There was a quadratic increase ($P < 0.05$) in HCW and decrease in carcass yield ($P <$

0.10). The maximum responses in ADG, HCW, and bone ash were estimated at 1.63:1, 1.11:1 to 1.60:1, and 1.25:1 analyzed Ca:P and at 1.75:1, 1.28:1 to 1.71:1, and 1.40:1 STTD Ca:STTD P, respectively. Expressing ADG on a STTD Ca:STTD P basis provided a more consistent estimate of the ideal Ca:P ratio among the two studies than analyzed Ca to analyzed P ratio. A STTD Ca:STTD P ratio between 1.75:1 to 1.82:1 can be used for 26- to 127-kg pigs that are fed diets adequate in STTD P with or without added phytase to maximize growth rate without reducing bone ash.

Key words: calcium, bone mineralization, finishing pigs, growth, modeling, phosphorus

INTRODUCTION

Calcium (**Ca**) and phosphorus (**P**) are the most abundant minerals in the pig and are involved in the lean tissue deposition, synthesis and maintenance of the skeletal structure, and in many other non-skeletal functions (Crenshaw, 2001; Berndt and Kumar, 2009; Kiarie and Nyachoti, 2010). Historically, swine diets are formulated with low margins of safety for P. Several reasons include an increase in dietary costs as well as an increase in P excretion if P is in excess (Fan et al., 2001; Maguire et al., 2005). Conversely, an abundant supply of Ca associated with its low cost and lack of environmental concerns likely lead to an increased risk for excess Ca in swine diets (Hall et al., 1991).

An excess or deficiency of either Ca or P may affect the utilization of the other mineral (Veum, 2010). Therefore, swine diets should supply not only the individual requirements of both minerals but also consider and adequate ratio between Ca and P (**Ca:P**). It is well established in the literature that a wide Ca:P ratio is detrimental to pig growth performance and bone mineralization, which is particularly evident when pigs are fed diets deficient or marginal in P (González-Vega et al., 2016a,b; Merriman et al., 2017; Wu et al., 2018). Moreover, an excess Ca

or a wide Ca:P ratio can promote the formation of insoluble Ca-phytate-P complexes in the small intestine and reduce the efficacy of exogenous phytases (Lei et al., 1994; Liu et al., 1998; Dersjant-Li et al., 2015).

A recent study has determined the requirement of standardized total tract digestible (**STTD**) P of growing finishing pigs (Vier et al., 2017). Therefore, the objective of this study was to determine the effects of feeding different Ca:P ratios in diets adequate in STTD P on growth performance, carcass characteristics, and bone mineralization of 26- to 127-kg pigs fed diets with or without phytase.

MATERIALS AND METHODS

The Kansas State University Institutional Animal Care and Use Committee (Manhattan, KS) approved all experimental procedures in this study.

Animals and Diets

Two studies were conducted at a commercial research-finishing site in southwestern Minnesota (New Horizon Farms, Pipestone, MN). The facilities were naturally ventilated and double-curtain-sided. Two barns were used containing 42 pens (3.05 × 5.49 m^2) each, with completely slatted concrete flooring and a deep pit for manure storage. Each pen was equipped with a 4-hole stainless steel, dry self-feeder (Thorp Equipment, Thorp, WI) and 1 cup waterer. The facilities were equipped with a computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN) capable of measuring and recording daily feed additions to individual pens. Thirteen barrows and fourteen gilts (PIC 359 × 1050, Genus PIC, Hendersonville, TN) were housed in each pen and were allowed ad libitum access to feed and water throughout the experiment.

A total of 1,134 pigs (initially 26.3 kg) in Exp. 1 and 1,215 pigs (initially 25.3 kg) in Exp. 2 were used in 110-d and 114-d growth trials, respectively. After placement in the finishing facility, pigs were fed a common diet containing 0.65% total Ca and 0.41% STTD P (0.54% total P) until the beginning of both trials. At d 0 of each trial, pens of pigs were sorted by average BW and randomly allotted to dietary treatments in a randomized complete block design with BW as the blocking factor. There were 27 pigs per pen with 7 replicate pens per treatment in Exp. 1 and 9 replicate pens per treatment in Exp. 2.

All treatment diets were manufactured at the New Horizon Farms Feed Mill in Pipestone, MN and fed in meal form. In Exp. 1, diets were formulated to 0:75:1, 1.00:1, 1.25:1, 1.50:1, 1.75:1, and 2.00:1 analyzed Ca:P ratios. In Exp. 2, diets were formulated to 0:75:1, 1.00:1, 1.25:1, 1.50:1, and 2.00:1 analyzed Ca:P ratios. Coefficients for STTD of P in feed ingredients were obtained from NRC (2012), and values for STTD of Ca were obtained from Stein et al. (2016). A weighted average of STTD Ca:STTD P ratios across dietary phases was calculated. In Exp. 1, these ratios were 0:96:1, 1.30:1, 1.65:1, 1.98:1, 2.32:1, and 2.67:1 STTD Ca:STTD P ratios across dietary treatments. In Exp. 2, these ratios were 0:95:1, 1.18:1, 1.40:1, 1.62:1, and 2.07:1 STTD Ca:STTD P ratios across dietary treatments. Prior to the experiments, 3 samples of the ingredients used in the diets that contained Ca and P were analyzed for Ca (method 985.01; AOAC International, 1990) and P (method 985.01; AOAC International, 1990) in duplicate (Ward Laboratories, Inc., Kearney, NE, Table 1). The average of the six lab results for each ingredient was used for diet formulation. The experimental diets were corn-soybean-meal–based and fed in 4 different phases (Tables 2 and 3). The diets were formulated to contain adequate STTD P across the dietary treatments in all phases based on the estimated requirement previously determined in this facility (Vier et al., 2017). Thus, formulated STTD P levels were

0.38, 0.33, 0.29, and 0.25% for phases 1, 2, 3, and 4, respectively, which represented 122% of NRC (2012) estimates. In Exp. 1, phase 1 diets were fed from d 0 to 28 (26.3- to 50.2-kg); phase 2 diets were fed from d 29 to 56 (50.2- to 78.2-kg); phase 3 diets were fed from d 57 to 85 (78.2- to 107.8-kg); and phase 4 diets were fed from d 86 to 110 (107.8- to 127.6-kg). In Exp. 2, phase 1 diets were fed from d 0 to 25 (25.3- to 44.6-kg); phase 2 diets were fed from d 26 to 58 (44.6- to 74.4-kg); phase 3 diets were fed from d 59 to 87 (74.4- to 103.0-kg); and phase 4 diets were fed from d 88 to 114 (103.0- to 126.6-kg).

The analyzed Ca:P ratios in Exp. 1 were achieved by increasing the amount of limestone at the expense of corn while maintaining monocalcium phosphate constant across treatments. No phytase was added to these diets. In Exp. 2, the diets contained 1,000 phytase units (**FYT**) of Ronozyme Hiphos 2500 (DSM Nutritional Products, Inc., Parsippany, NJ) with assumed release values of 0.15% available P, 0.132% STTD P, 0.144% total Ca, and 0.096% STTD Ca. Therefore, the STTD P and STTD Ca concentrations included the expected phytase release of 0.132% STTD P and 0.096% STTD Ca. The analyzed Ca:P ratios to form the dietary treatments represent the analyzed Ca and P in feed ingredients, without including any Ca release from phytase. The treatments were achieved by increasing the amount of limestone at the expense of corn while maintaining monocalcium phosphate constant across treatments. In both experiments, beef tallow was included in diets to equalize net energy across dietary treatments without giving phytase any energy value in the second experiment.

Pens of pigs were weighed, and feed disappearance was recorded on d 0, 28, 56, 85, and 110 in Exp. 1 and on d 0, 25, 58, 87, and 114 in Exp. 2 to determine average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**). Removals and mortality were

recorded, and the weight gain and feed consumption were accounted for in the analysis of the data.

Carcass and Bone Data Colletcion

The 2 heaviest pigs in each pen were selected, weighed, and marketed on d 86 in Exp. 1 and d 99 in Exp. 2 according to standard farm protocol. These pigs were included in calculation of pen growth performance, but not carcass characteristics. On d 110 and 114 in Exp. 1 and Exp. 2, respectively, final pen weights were taken, and one barrow and one gilt were identified to represent the mean individual pig weight of the pen, tattooed with a pen identification and marked for bone data collection. These pigs were transported to a commercial abattoir in northwest Iowa (Natural Food Holdings, Sioux Center, IA) for processing and bone collection. Following processing, the left front feet were separated at the junction of carpals and radius and ulna and individually placed in a zip-lock plastic bag with a permanent identification tag within the bag. These feet were transferred on dry ice to the Kansas State University Swine Laboratory and stored at -20℃ until analysis of bone mineral content.

The remaining pigs were individually tattooed with the specific pen identity on the shoulder to allow for carcass measurements to be recorded on a pen basis. These pigs were transported to a commercial packing plant in southwestern Minnesota (JBS Swift and Company, Worthington, MN) for processing and carcass data collection. Carcass measurements included hot carcass weight (**HCW**), loin depth, backfat depth, and percentage lean. Fat depth and loin depth were measured with an optical probe inserted between the third and fourth last rib (counting from the ham end of the carcass) at a distance approximately 7 cm from the dorsal midline. Percentage carcass yield was calculated by dividing the average pen HCW collected at the plant by the average final live weight at the farm before transport.

Bone Ash Analysis

After thawing overnight, the feet were autoclaved for 1 h at 121℃. The third and fourth metacarpals of each foot were removed. These bones were cleaned of extraneous soft tissue, and refrozen. The third metacarpal was dried at ambient temperature for 24 h and cut in half and weighed. They were wrapped in cheesecloth to keep their tag identification number and defatted by petroleum ether using a Soxhlet apparatus for 7 d. De-fated metacarpals were placed in a drying oven at 105℃ for 24 h to determine dry fat-free weight. Bones were then ashed in a muffle furnace at 600℃ for another 24 h to determine percentage ash. Ash is expressed as a percentage of dried fat-free bone weight.

Chemical Analysis

Representative diet samples were obtained from 6 feeders of each treatment approximately 3 d after the beginning and 3 d before the end of the phase and delivered to the Kansas State University Swine Laboratory, Manhattan, KS, and stored at -20°C until analysis. Samples of the diets were combined within dietary treatment, and a composite sample from each treatment was analyzed in duplicate (Ward Laboratories, Inc., Kearney, NE, Tables 5 and 6). Samples were analyzed for Ca and P (method 985.01; AOAC International, 1990).

Statistical Analysis

Experimental growth data were analyzed as a randomized complete block design with block as a random effect and pen as the experimental unit. The study was structured as a split-plot design in a randomized complete block design for the bone data for both trials. The whole-plot treatments included the different Ca:P ratios. Within each of the dietary treatments, there was a one-way treatment structure with gender as the factor level. A random effect of block by treatment was used to identify the pair of pigs (one barrow and one gilt) within each pen as the

experimental unit for gender. The two-way interaction between dietary treatments and sex was tested. Response variables were analyzed using generalized linear and non-linear mixed models. Polynomial contrasts were implemented to evaluate the functional form of the dose response to increasing dietary Ca:P ratios on ADG, ADFI, G:F, BW, percentage bone ash, HCW, carcass yield, carcass backfat, carcass fat-free lean, and carcass loin depth. Backfat depth, loin depth, and percentage lean were adjusted to a common HCW. The Kenward–Roger method was used to adjust the denominator degrees of freedom and correct the standard errors for bias (Littell, et al., 2006). The coefficients for the unequally spaced linear and quadratic contrasts in Exp. 2 were derived using the IML procedure in SAS (Version 9.3, SAS Institute Inc., Cary, NC). Statistical models were fit using GLIMMIX procedure of SAS. Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

In addition, the effects of the analyzed Ca:P ratios and STTD Ca:STTD P ratios on overall ADG, ADFI, G:F, HCW, and percentage bone ash were fit using procedures outlined by Gonçalves et al. (2016) . Briefly, models were expanded to account for heterogeneous residual variances when needed. Competing statistical models included a linear (**LM**), quadratic polynomial (**QP**), broken-line linear (**BLL**), and broken-line quadratic (**BLQ**). Dose response models were compared based on the Bayesian information criterion (**BIC**), where the smaller the value, the better (Milliken and Johnson, 2009). A decrease in BIC greater than 2 was considered a significant improvement in model fit. The 95% confidence interval of the estimated requirement to reach maximum performance or to reach plateau performance was computed. Results reported correspond to inferences based on the best fitting models.

RESULTS

Chemical Analysis

In both Exp.1 and Exp. 2, the analyzed Ca and P contents of experimental diets were consistent with formulated values (Tables 4 and 5). In Exp. 1, the average Ca:P ratio across the four phases were consistent with formulated values at 0.77:1, 1.02:1, 1.25:1, 1.55:1, 1.77:1, and 2.07:1 for the 0.75:1, 1.00:1, 1.25:1, 1.50:1, 1.75:1, and 2.00:1 treatments, respectively. In Exp. 2, the average Ca:P ratio across the four phases were also similar to formulated values at 0.78:1, 0.97:1, 1.32:1, 1.47:1, and 2.06:1 for the 0.75:1, 1.00:1, 1.25, 1.50, and 2.00:1 treatments, respectively.

Experiment 1

During the grower period, which corresponds to phases 1 and 2 (d 0 to 56), there was no evidence of differences ($P > 0.05$) in ADG as the ratio between Ca and P increased (Table 6). In contrast, increasing Ca:P ratio resulted in a marginal increase (linear, $P < 0.10$) in ADFI, and in an improvement (quadratic, $P < 0.05$) in G:F. The greatest improvement in G:F occurred as the analyzed Ca:P ratio increased from 0.75:1 to 1.00:1, which is equivalent to increasing STTD Ca:STTD P from 0.96:1 to 1.30:1, and then it worsened at the highest ratio of 2.00:1 analyzed Ca:P (2.67:1 STTD Ca:STTD P). During the finisher period, which corresponds to phases 3 and 4 (d 56 to 110), increasing the Ca:P ratio resulted in an increase (quadratic, $P < 0.05$) in ADG driven by an increase (quadratic, $P < 0.05$) in ADFI. The greatest improvement occurred as the analyzed Ca:P ratio increased from 0.75:1 to 1.25:1 for ADG, which represents an increase in STTD Ca:STTD P ratio from 0.96:1 to 1.65:1, and from 0.75:1 to 1.00:1 analyzed Ca:P or 0.96:1 to 1.30:1 STTD Ca:STTD P for ADFI, and then started to decrease at the higher ratios. Overall, increasing Ca:P ratio increased (quadratic, $P < 0.05$) ADG, ADFI, and final BW. The greatest

increase was observed as the ratio increased from 0.75:1 to 1.25:1 analyzed Ca:P (0.96:1 to 1.65:1 STTD Ca:STTD P), decreasing at the higher ratios. Feed efficiency was relatively similar from an analyzed Ca:P ratio of 0.75:1 to 1.75:1 (0.96:1 to 2.32:1 STTD Ca:STTD P), and started to worsen (quadratic, $P < 0.05$) at the highest ratios.

Homogeneous variance was used for ADG and heterogeneous variance was used for ADFI and G:F models (Tables 8 and 9). The QP model estimated the analyzed Ca:P ratio for maximum ADG at 1.38:1 (95% CI: [1.00:1, 1.75:1]). Similarly, the QP model had the best fit for ADG as a function of STTD Ca:STTD P (Figure 1A), estimating the maximum mean ADG at 1.82:1 (95% CI: [1.30:1, 2.31:1]). The QP model estimated the maximum mean ADFI at 1.49:1 analyzed Ca:P ratio (95% CI: [0.90:1,>2.00:1]). Both the QP and BLL had similar fit to the ADFI data as a function of increasing STTD Ca:STTD P, estimating the maximum mean ADFI at 1.97:1 (95% CI: [1.30:1,>2.67:1]) and 1.30:1 (95% CI: [0.93:1, 1.67:1]), respectively. The analyzed Ca:P ratio for maximum mean G:F was estimated at 1.29:1 (95% CI: [<0.75:1,>2.00:1]) based on the QP model. Also based on the QP model, the STTD Ca:STTD P ratio for maximum mean G:F was estimated at 1.69:1 (95% CI: [<0.96:1,>2.67:1]).

For carcass characteristics, HCW increased (quadratic, $P < 0.05$) as the analyzed Ca:P ratio increased up to 1:25:1 and the STTD Ca:STTD P ratio increased up to 1.65:1, and started to decrease thereafter (Table 6). Percentage carcass yield decreased (linear, $P < 0.10$), with the greatest decrease as analyzed Ca:P ratio increased to 1.50:1 (1.98:1 STTD Ca:STTD P), with no further changes at the higher ratios. There was also a marginally significant response (quadratic, $P < 0.10$) in loin depth, with the greatest improvement occurring up to 1:50:1 analyzed Ca:P ratio, which represents 1.98:1 STTD Ca:STTD P ratio. No statistically significant differences ($P > 0.10$) were observed for carcass backfat and fat-free lean measurements. Heterogeneous

variance was used to model HCW, with the QP model best fitting the HCW data (Tables 8 and 9). The QP model estimated the maximum mean HCW at 1.25:1 analyzed Ca:P ratio (95% CI: [0.86:1,1.72:1]) and at 1.64:1 STTD Ca:STTD P ratio (95% CI: [1.07:1,2.31:1]).

For bone mineralization, the two-way interaction between dietary treatment and gender was tested and no evidence ($P > 0.10$) for a significant interaction was observed (Table 6). There was also no evidence ($P > 0.10$) for a significant gender effect on percentage bone ash (62.1 and 62.0% for barrows and gilts, respectively). Bone mineralization increased (quadratic, $P < 0.05$) with increasing Ca and P ratio. The greatest improvement in percentage bone ash was observed as analyzed Ca:P ratio increased from 0.75:1 to 1.25:1 or from 0.96:1 to 1.65:1 STTD Ca:STTD P, with diminishing returns thereafter. Heterogeneous variance was used to model percentage bone ash, with the QP model representing the best fit (Tables 8 and 9). The maximum mean percentage bone ash as a function of analyzed Ca:P ratio was estimated at 1.93:1 (95% CI: [1.40:1,>2.00:1]), with 99.8% of maximum bone ash achieved at 1.63:1 analyzed Ca:P. The maximum mean percentage bone ash as a function of STTD Ca:STTD P was estimated at 2.57:1 (95% CI: [1.85:1,>2.67:1]), with 99.5% of maximum bone ash achieved at 1.82:1 STTD Ca:STTD P.

Experiment 2

During the grower period, which corresponds to phases 1 and 2 (d 0 to 58), there was a marginal increase (quadratic, $P < 0.10$) in ADG with the greatest gain observed at 1.50:1 analyzed Ca:P ratio, which is equivalent to 1.62:1 STTD Ca:STTD P, with no improvements thereafter (Table 7). However, there was no evidence ($P > 0.10$) of differences in ADFI and G:F due to increasing Ca:P ratio. During the finisher period, which corresponds to phases 3 and 4 (d 59 to 114), increasing Ca:P ratio resulted in an increase (quadratic, $P < 0.05$) in ADG driven by

an increase (quadratic, $P < 0.05$) in ADFI. The greatest improvements in ADG and ADFI were observed as the ratio increased to 1.25:1 analyzed Ca:P, which is equivalent to 1.40:1 STTD P, with no further benefits at higher ratios. Feed efficiency improved (linear, $P < 0.05$) as the ratio increased up to the highest ratio of 2.00:1 analyzed Ca:P (2.07:1 STTD Ca:STTD P).

For overall growth performance (d 0 to 114), increasing Ca:P ratio increased (quadratic, $P < 0.05$) ADG and final BW. The greatest increase in both criteria was observed as the ratio increased from 0.75:1 to 1.50:1 analyzed Ca:P (0.95:1 to 1.62:1 STTD Ca:STTD P), with no improvements thereafter. Similarly, there was a marginal increase (quadratic, $P < 0.10$) in ADFI, with the greatest intake observed at an analyzed Ca:P ratio of 1.50:1 or at an STTD Ca:STTD P ratio of 1.62:1. Feed efficiency improved (linear, $P < 0.05$) with increasing the Ca:P ratio, with the greatest improvement, however, as the analyzed Ca:P ratio increased from 0.75:1 to approximately 1.00:1 and 1.25:1 or from 0.95:1 to approximately 1.18:1 and 1.40:1 STTD Ca:STTD P.

Homogeneous variance was used for G:F models and heterogeneous variance was used for ADG and ADFI models (Tables 8 and 9). The best fitting model for ADG was the QP model. The analyzed Ca:P ratio for maximum ADG was estimated at 1.63:1 (95% CI: [1.25:1, >2.00:1]). The STTD Ca:STTD P ratio for maximum ADG (Figure 1B) was estimated at 1.75:1 (95% CI: [1.40:1, >2.07:1]). The best fitting model for ADFI was the LM, which estimated the maximum mean ADFI at greater than 2.00:1 analyzed Ca:P ratio and greater than 2.07:1 STTD Ca:STTD P ratio. Broken-line linear model provided the best fit for G:F, with the breakpoint observed at 1.05:1 analyzed Ca:P ratio (95% CI: [0.81:1,1.30:1]). As a function of STTD Ca:STTD P, G:F increased linearly as the ratio increased based on the LM model.

For carcass characteristics, HCW increased (quadratic, $P < 0.05$) as the analyzed Ca:P ratio increased up to 1:50:1 and up to 1.61:1 STTD Ca:STTD P ratio, with no further benefits thereafter (Table 7). Percentage carcass yield decreased (quadratic, $P < 0.10$) from 0.75:1 analyzed Ca:P ratio to 1.25:1 and from 0.95:1 STTD Ca:STTD P ratio to 1.40:1, slightly increasing at higher ratios. No evidence of differences ($P > 0.10$) was observed for backfat depth, fat-free lean, and loin depth measurements. Heterogeneous variance was used for HCW, with the QP and BLL models having similar fit (Tables 8 and 9). The QP model estimated the maximum mean HCW at 1.60:1 analyzed Ca:P ratio (95% CI: [1.14:1,>2.00:1]), while the BLL plateau was estimated at 1.11:1 analyzed Ca:P ratio (95% CI: [0.87:1,1.36:1]). The QP model estimated the maximum mean HCW at 1.71:1 STTD Ca:STTD P ratio (95% CI: [1.28:1,>2.07:1]), while the BLL plateau was estimated at 1.28:1 STTD Ca:STTD P ratio (95% CI: [1.06:1,1.50:1]).

For bone mineralization, the two-way interaction between dietary treatment and gender was tested and no evidence ($P > 0.10$) for significant interaction was observed. There was a marginal significant gender effect ($P < 0.10$) on percentage bone ash, with barrows having greater bone mineralization than gilts (61.6 and 61.3% for barrows and gilts, respectively). Percentage bone ash increased (quadratic, $P < 0.05$) with increasing Ca:P ratio (Table 7). The greatest improvement in percentage bone ash was observed as analyzed Ca:P ratio increased from 0.75:1 to 1.25:1 and as the STTD Ca:STTD P ratio increased from 0.95:1 to 1.40:1, with no further increase thereafter. Heterogeneous variance was used for percentage bone ash, with the BLL being the best fitting model (Tables 8 and 9). The plateau for maximum mean percentage bone ash was estimated at 1.25:1 analyzed Ca:P ratio (95% CI: [1.10:1, 1.40:1]) and at 1.40:1 STTD Ca:STTD P ratio (95% CI: [1.26:1, 1.54:1]).

DISCUSSION

An abundance or deficiency of either Ca or P may influence the absorption and utilization of the other mineral, as both minerals are interdependent (Crenshaw, 2001). As an example, it has been extensively demonstrated that an excess of Ca is detrimental to pig growth performance, and this effect is exacerbated when diets are marginal or low in P (Reinhart and Mahan, 1986; González-Vega et al., 2016b; Wu et al., 2018). This effect may occur through a P digestibility reduction (Stein et al., 2011) when diets contain a high Ca concentration. This leads to the formation of Ca-P insoluble complexes in the gastrointestinal tract which leads to decreased P digestion and absorption (Brink et al., 1992; Heaney and Nordin, 2002; González-Vega and Stein, 2014). The detrimental effects of high Ca diets on pig growth performance was alleviated by increasing the dietary concentration of STTD P. These observations emphasize the importance of considering an appropriate ratio between Ca and P when formulating diets for pigs to ensure optimum absorption of both minerals and optimum performance of pigs.

In Exp. 1, we observed that at high concentrations of Ca, corresponding to an analyzed Ca:P ratio greater than 1.38:1 or an STTD Ca:STTD P ratio greater than 1.82:1, ADG is reduced mainly due to a reduction in feed intake. Similar observations were described by Sørensen et al. (2018). Results from this experiment are in agreement with a study with added phytase in 9- to 23-kg nursery pigs where narrowing the ratio of total Ca:total P from 2.00:1 to 1.20:1 resulted in improvements in growth performance, independent of the dietary P concentration (Qian et al., 1996). Wu et al. (2018) also observed that excess Ca impairs ADG, ADFI, and G:F of nursery pigs fed diets deficient in P, and that these negative effects are ameliorated by providing more P in the diet. These detrimental effects of high Ca or wide Ca:P ratios were also reported by González-Vega et al. (2016a) with 11- to 25-kg pigs, González-Vega et al. (2016b) with 25- to

50-kg pigs, and Merriman et al. (2017) with 100- to 130-kg pigs that were fed diets with no added phytase. Interestingly, the negative effects of increased dietary Ca concentration in the present study were observed in diets containing 122% of NRC (2012) STTD P requirement estimates across phases. Therefore, the current study demonstrated that the ratio between Ca and P is important even when dietary P concentration is provided in excess of those suggested by the NRC (2012).

In Exp. 2, we observed that growth rate, feed intake, and feed efficiency are improved with increasing Ca:P ratio. Differently than the results from Exp.1, growth performance wasn't reduced at the highest Ca level or the highest analyzed Ca:P and STTD Ca:STTD P ratios. Due to the addition of phytase in these diets, the analyzed Ca levels were approximately 30% below the analyzed Ca concentration in Exp. 1. This explains why pigs were able to tolerate a wider analyzed Ca:P ratio when phytase was added to the diets. Moreover, lower growth rate and feed intake were observed in both experiments with low Ca diets, which corresponded to narrow analyzed Ca:P ratios. The study conducted by Lagos et al. (2019) suggested that binding of Ca due to an abundance of P can also occur as demonstrated by a reduced ADG in diets with excess P and low Ca compared to higher Ca concentration in their study. This observation from the current study is in contrast to results from González-Vega et al., (2016b) and Merriman et al., (2017), where it was not possible to decrease pig growth performance through a reduction in dietary Ca concentration. As opposed to our study that evaluated varying analyzed Ca:P ratios throughout the entire grower and finisher periods, the aforementioned studies were short term experiments. Thus, pigs may have had enough bone mineral reserves to supply Ca and P and alleviate their deficiencies during the aforementioned experiments.

Recent research has demonstrated that the addition of phytase can improve the STTD of Ca in feed ingredients and, therefore, should be considered when formulating diets for pigs (González-Vega et. al., 2015; Stein et al., 2016). Observations from our study demonstrated that, as opposed to analyzed Ca:P, the ratio of STTD Ca:STTD P is more accurate when comparing results between the two studies with or without added phytase. As an example, growth rate was maximized at 1.38:1 in Exp. 1 and at 1.63:1 in Exp. 2 on an analyzed Ca to analyzed P basis. However, growth rate was maximized at 1.82:1 in Exp. 1 and at 1.75:1 in Exp. 2 on an STTD Ca to STTD P basis. This further illustrates the value of comparing Ca and P nutrient values on a STTD basis. Moreover, these results are in agreement with data from Lagos et al. (2019) that suggested a STTD Ca:STTD P ratio greater than 1.50:1 is needed to maximize growth in 50- to 85-kg pigs if the STTD P exceeds NRC (2012) estimates, which was the case of the current study.

Negative effects of high Ca levels and wide total Ca:P ratios on efficacy of phytase have also been reported (Qian et al., 1996). According to the authors, a reduction in the dietary total Ca:P from 2.0:1 to 1.2:1 resulted in an improvement of approximately 16% in the releasing ability of phytase to increase growth and bone performance. This detrimental effect on phytase efficacy is consistent to other observations in weanling pigs (Lei et al., 1994) and broilers (Shirley and Edwards, 2002). The binding capacity of phytate to Ca resulting in insoluble complexes resistant to phytase hydrolysis is among the potential mechanisms of the detrimental effects of high Ca on phytase efficacy (Selle et al., 2009; Dersjant-Li et al., 2015). Other mechanisms include a competitive inhibition of the active sites of the enzyme, and the high acid binding capacity of limestone and monocalcium phosphate (Selle et al., 2009; Dersjant-Li et al., 2015). However, in this study the analyzed Ca concentration in diets containing added phytase

were possibly not high enough to elicit such detrimental effects on phytase activity. Moreover, Qian et al. (1996) observed that the high Ca or wide Ca:P ratio effects on efficacy of phytase were more detrimental at low P concentrations.

The greatest carcass weight occurred at a ratio of 1.25:1 analyzed Ca:P and 1.65:1 STTD Ca:STTD P in Exp. 1 and at a ratio of 1.50:1 analyzed Ca:P and 1.62:1 STTD Ca:STTD P in Exp. 2. Similarly, results from Hanni et al. (2005) have shown that increasing the ratio between Ca and P improved HCW, with detrimental effects at a ratio greater than 1.50:1 total Ca:P. Our results are in accordance with findings from Liu et al., (1998) and Hanni et al. (2005) in which backfat depth, fat-free lean, and loin depth were not affected by the ratio between Ca and P. There was, however, a marginal curvilinear response in loin depth up to 1.50:1 analyzed Ca:P ratio or 1.62:1 STTD Ca:STTD P ratio in Exp. 2. Percentage carcass yield decreased as the Ca:P ratio increased. There is not a clear explanation for the reduction in yield, but this observation was consistent among Exp. 1 and 2. In contrast, other studies have indicated no evidence for influence of Ca and P ratios on carcass yield (Liu et al., 1998; Hanni et al., 2005).

Bone mineral deposition is dependent on the presence of both Ca and P, and they accumulate in a constant Ca:P ratio of 2.2:1 in the form of hydroxyapatite (Crenshaw, 2001). Findings from several studies suggest that pigs are able to deposit significantly more Ca and P in bones than the amount needed to optimize pig growth performance (González-Vega et al., 2016a,b; Merriman et al., 2017; Lagos et al., 2019). In our study, however, increasing Ca and P to maximize growth rate resulted in 99.5 to 99.8% and 100% of maximum bone mineralization in Exp. 1 and Exp. 2, respectively. This observation contradicts the expectation that concentrations of Ca and P to maximize synthesis of skeletal tissue is greater than the amount required to maximize muscle growth. Therefore, results from our study suggest that if P exceeds

the NRC (2012) requirements, Ca concentration is not so important to optimize bone mineralization. Different from Exp. 1, we observed a marginally greater percentage bone ash for barrows compared to gilts in Exp. 2. A greater bone mineralization in barrows compared to gilts have been reported in a study evaluating increasing levels of STTD P (Vier et al., 2017), whereas other studies have reported no evidence for differences (Crenshaw et al., 1981; Hanni et al., 2005).

Taken together, results from this study highlighted the importance of considering an appropriate ratio between Ca and P when formulating diets for pigs, even when dietary P concentration is provided in excess of that suggested by the NRC (2012). Our data suggests that the analyzed Ca:P ratio to optimize growth performance, HCW, and bone mineralization ranged from 1.25:1 to 1.93:1 and 1.05:1 to 1.63:1 in diets without or with phytase, respectively. A more consistent ratio among experiments was observed for ADG on a STTD Ca:STTD P basis, with estimated requirements at 1.75:1 and 1.82:1 in diets without and with phytase, respectively. Bone mineralization was maximized at 2.57:1 and 1.40:1 STTD Ca:STTD P ratio. However, approximately 99.5 to 100% of maximum bone ash was captured at the ratios needed to maximize growth rate. In conclusion, a ratio between 1.75:1 to 1.82:1 STTD Ca:STTD P can be used in diet formulation of 26- to 127-kg pigs that are fed diets adequate in STTD P with or without added phytase to optimize growth rate without reducing bone mineralization. These data demonstrate the value of comparing Ca and P nutrient values on a STTD basis.

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Table 3.1 Analyzed Ca and P concentrations in feed ingredients (as-fed basis)[1]

Item	Ca, %	P, %
Corn	0.03	0.22
Soybean meal, 46,5% crude protein	0.51	0.65
Monocalcium P (21% P)	15.23	18.78
Limestone	34.64	0.07
Vitamin and trace mineral premix	6.21	0.02

[1]A total of six samples of each ingredient were submitted to Ward Laboratories, Inc. (Kearney, NE) and were analyzed in duplicate for Ca and P concentration. Average values were reported and were used in diet formulation.

Table 3.2 Diet composition for Phases 1 to 4 diets (Exp. 1; as-fed basis)[1]

Item	Phase 1	Phase 2	Phase 3	Phase 4
Ingredient, %				
Corn	67.09 – 63.12	75.42 – 71.94	80.49 – 77.33	80.91 – 78.02
Soybean meal, 46.5% CP	29.97 – 30.25	21.79 – 22.04	16.88 – 17.10	16.82 – 17.02
Beef tallow	0.50 – 1.95	0.50 – 1.75	0.50 – 1.65	0.50 – 1.55
Monocalcium phosphate, 21% P	1.25	1.08	0.90	0.68
Limestone	0.20 – 2.44	0.25 – 2.23	0.28 – 2.06	0.29 – 1.92
Sodium chloride	0.35	0.35	0.35	0.35
L-lysine HCl	0.30	0.30	0.30	0.23
DL-methionine	0.09	0.06	0.04	0.01
L-threonine	0.10	0.09	0.10	0.08
L-tryptophan	0.01	0.01	0.02	0.01
Vitamin and trace mineral premix[2]	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Standardized ileal digestible amino acids, %				
Lysine	1.15	0.95	0.83	0.77
Isoleucine:lysine	63	62	61	66
Leucine:lysine	132	139	146	157
Methionine:lysine	32	32	31	30
Methionine and cysteine:lysine	56	57	58	59
Threonine:lysine	62	63	65	67
Tryptophan:lysine	18.9	18.6	18.7	18.8
Valine:lysine	69	69	70	75
Total lysine, %	1.29	1.07	0.94	0.88
Net energy, kcal/kg	2,469	2,520	2,553	2,557
Crude protein, %	19.4	16.0	14.1	13.9
Calcium, %	0.48 – 1.27	0.43 – 1.12	0.38 – 1.01	0.35-0.93
STTD Ca[3], %	0.36 – 0.97	0.32 – 0.85	0.28 – 0.76	0.25 – 0.70
Phosphorus, %	0.64	0.57	0.51	0.47
STTD P[4], %	0.38	0.33	0.29	0.25
Available phosphorus, %	0.32	0.28	0.24	0.19

Calcium:phosphorus	0.75:1 – 2.00:1	0.75:1 – 2.00:1	0.75:1 – 2.00:1	0.75:1 – 2.00:1
STTD Ca:STTD P	0.95:1 – 2.56:1	0.95:1 – 2.60:1	0.96:1 – 2.68:1	0.99:1 – 2.80:1

[1] Treatments were formulated to 0.75:1, 1.00:1, 1.25:1, 1.50:1, 1.75:1, and 2.00:1 analyzed calcium to analyzed phosphorus ratios across dietary phases. These represent a weighted average of 0.96:1, 1.30:1, 1.65:1, 1.98:1, 2.32:1, and 2.67:1 STTD Ca:STTD P ratios. Treatments were achieved with the addition of limestone at the expense of corn. Beef tallow was added to balance net energy across treatments. Phase 1 diets were fed from d 0 to 28 (26.3- to 50.2-kg BW), Phase 2 from d 28 to 56 (50.2- to 78.2-kg BW). Phase 3 from d 56 to 85 (78.2- to 107.8-kg BW) and Phase 4 from d 85 to 110 (107.8 to 127.6 kg BW).

[2] Provided per kg of premix: 8,818,490 IU vitamin A; 1,102,311 IU vitamin D; 35,273 IU vitamin E; 3,527.4 mg vitamin K; 30.9 mg vitamin B12; 39,683 mg niacin; 22,046 mg pantothenic acid; 6,614 mg riboflavin, 165 g Zn from Zn sulfate; 165 g Fe from iron sulfate; 40 g Mn from manganese oxide; 17 g Cu from copper sulfate; 0.3 g I from calcium iodate; 0.3 g Se from sodium selenite.

[3] STTD Ca = standardized total tract digestible calcium.

[4] STTD P = standardized total tract digestible phosphorus.

Table 3.3 Diet composition for Phases 1 to 4 diets (Exp. 2; as-fed basis)[1]

Item	Phase 1	Phase 2	Phase 3	Phase 4
Ingredient, %				
Corn	67.85 – 64.83	76.19 – 73.60	81.22 – 78.99	81.56 – 79.50
Soybean meal, 46.5% CP	29.91 – 30.13	21.74 – 21.92	16.83 – 16.99	16.77 – 16.92
Beef tallow	0.50 – 1.60	0.50 – 1.45	0.50 – 1.30	0.50 – 1.25
Monocalcium phosphate, 21% P	0.48	0.30	0.15	-
Limestone	0.23 – 1.94	0.28 – 1.73	0.31 – 1.58	0.32 – 1.48
Sodium chloride	0.35	0.35	0.35	0.35
L-lysine HCl	0.30	0.30	0.30	0.23
DL-methionine	0.09	0.06	0.04	0.01
L-threonine	0.10	0.09	0.10	0.08
L-tryptophan	0.01	0.01	0.02	0.01
Phytase[2]	0.04	0.04	0.04	0.04
Vitamin and trace mineral premix[3]	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Standardized ileal digestible amino acids, %				
Lysine	1.15	0.95	0.83	0.77
Isoleucine:lysine	63	62	61	66
Leucine:lysine	132	140	146	158
Methionine:lysine	32	32	31	30
Methionine and cysteine:lysine	56	57	58	59
Threonine:lysine	62	63	65	67
Tryptophan:lysine	18.9	18.6	18.7	18.8
Valine:lysine	69	69	70	75
Total lysine, %	1.29	1.07	0.94	0.88
Net energy, kcal/kg	2,489	2,540	2,571	2,575
Crude protein, %	19.4	16.1	14.1	14.0
Analyzed calcium, %	0.37 – 0.97	0.31 – 0.82	0.27 – 0.72	0.25 – 0.66
Total calcium with phytase, %	0.51 – 1.11	0.45 – 0.96	0.42 – 0.86	0.39 – 0.80
STTD Ca[4] with phytase, %	0.35 – 0.78	0.31 – 0.67	0.27 – 0.59	0.26 – 0.55
Phosphorus, %	0.49	0.41	0.36	0.33

STTD P[5] with phytase, %	0.38	0.33	0.29	0.26
Available phosphorus, %	0.32	0.27	0.24	0.21
Analyzed calcium:phosphorus	0.75:1 – 2.00:1	0.75:1 – 2.00:1	0.75:1 – 2.00:1	0.75:1 – 2.00:1
STTD Ca:STTD P	0.93:1 – 2.07:1	0.94:1 – 2.06:1	0.95:1 – 2.06:1	0.98:1 – 2.09:1

[1] Treatments were formulated to 0.75:1, 1.00:1, 1.25:1, 1.50:1, and 2.00:1 analyzed calcium to analyzed phosphorus ratios across dietary phases. These represent a weighted average of 0.95:1, 1.18:1, 1.40:1, 162:1and 2.07:1 STTD Ca:STTD P ratios. Treatments were achieved with the addition of limestone at the expense of corn. Beef tallow was added to balance net energy across treatments. Phase 1 diets were fed from d 0 to 25 (25.3- to 44.6-kg BW), phase 2 from d 26 to 58 (44.6- to 74.4-kg BW), phase 3 from d 59 to 87 (64.4- to 103.0-kg BW), and phase 4 from d 88 to 114 (103.0- to 126.6-kg BW).

[2] Phytase (Ronozyme HiPhos, DSM Nutritional Products, Parsippany, NJ) was included at 1,000 FYT/kg releasing an assumed 0.15% aP, 0.132% STTD P, 0.144% total Ca, and 0.096% STTD Ca.

[3] Provided per kg of premix: 8,818,490 IU vitamin A; 1,102,311 IU vitamin D; 35,273 IU vitamin E; 3,527.4 mg vitamin K; 30.9 mg vitamin B12; 39,683 mg niacin; 22,046 mg pantothenic acid; 6,614 mg riboflavin, 165 g Zn from Zn sulfate; 165 g Fe from iron sulfate; 40 g Mn from manganese oxide; 17 g Cu from copper sulfate; 0.3 g I from calcium iodate; 0.3 g Se from sodium selenite.

[4] STTD Ca = standardized total tract digestible calcium.

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

Table 3.4 Chemical analysis of experimental diets (Exp.1; as-fed-basis)[1]

Item	Analyzed Ca:P ratio: STTD Ca:STTD P rati	0.75:1 0.96:1	1.00:1 1.30:1	1.25:1 1.65:1	1.50:1 1.98:1	1.75:1 2.32:1	2.00:1 2.67:1
Calcium (Ca), %							
Phase 1		0.49	0.74	1.08	1.24	1.37	1.74
Phase 2		0.62	0.97	0.85	1.12	1.31	1.33
Phase 3		0.47	0.68	0.74	0.95	1.03	1.33
Phase 4		0.41	0.48	0.73	0.88	1.12	1.13
Phosphorus (P), %							
Phase 1		0.70	0.80	0.83	0.76	0.80	0.78
Phase 2		0.70	0.73	0.71	0.70	0.72	0.67
Phase 3		0.64	0.69	0.67	0.68	0.68	0.64
Phase 4		0.55	0.58	0.52	0.56	0.55	0.57
Ca:P ratio							
Phase 1		0.70:1	0.93:1	1.30:1	1.63:1	1.71:1	2.23:1
Phase 2		0.89:1	1.33:1	1.20:1	1.60:1	1.82:1	1.99:1
Phase 3		0.73:1	0.99:1	1.10:1	1.40:1	1.51:1	2.08:1
Phase 4		0.75:1	0.83:1	1.40:1	1.57:1	2.04:1	1.98:1

[1] Representative samples of treatment diets were taken from 6 feeders per dietary treatment 3 d after the beginning and 3 d before the end of the phase and stored at -20°C. After blending, subsamples were submitted to Ward Laboratories, Inc. (Kearney, NE) and Midwest Laboratories (Omaha, NE) for analyses in duplicate. Values represent the average across laboratories.

[2] Coefficients for standardized total tract digestibility (STTD) of P were obtained from NRC (2012), while values for STTD Ca in feed ingredients were obtained from Stein (2016). These represent a weighted average of the ratio between STTD Ca:STTD P across treatments for the four dietary phases.

Table 3.5 Chemical analysis of experimental diets (Exp.2; as-fed-basis)[1,2]

Item	Analyzed Ca:P ratio: STTD Ca:STTD P rati	0.75:1 0.95:1	1.00:1 1.18:1	1.25:1 1.40:1	1.50:1 1.62:1	2.00:1 2.07:1
Calcium (Ca), %						
Phase 1		0.47	0.53	0.70	0.84	0.97
Phase 2		0.33	0.38	0.56	0.69	0.87
Phase 3		0.30	0.41	0.54	0.62	0.87
Phase 4		0.27	0.36	0.50	0.48	0.81
Phosphorus (P), %						
Phase 1		0.54	0.53	0.53	0.54	0.52
Phase 2		0.45	0.45	0.46	0.47	0.46
Phase 3		0.39	0.41	0.39	0.41	0.40
Phase 4		0.36	0.35	0.38	0.35	0.36
Ca:P ratio						
Phase 1		0.87:1	1.00:1	1.32:1	1.56:1	1.87:1
Phase 2		0.73:1	0.84:1	1.22:1	1.47:1	1.89:1
Phase 3		0.77:1	1.00:1	1.38:1	1.51:1	2.18:1
Phase 4		0.75:1	1.03:1	1.32:1	1.37:1	2.25:1

[1] Representative samples of treatment diets were taken from 6 feeders per dietary treatment 3 d after the beginning and 3 d before the end of the phase and stored at -20°C. After blending, subsamples were submitted to Ward Laboratories, Inc. (Kearney, NE) and Midwest Laboratories (Omaha, NE) for analyses in duplicate. Values represent the average across laboratories.

[2] Coefficients for standardized total tract digestibility (STTD) of P were obtained from NRC (2012), while values for STTD Ca in feed ingredients were obtained from Stein et al. (2016). These represent a weighted average of the ratio between STTD Ca:STTD P across treatments for the four dietary phases.

Table 3.6 Least square means for growth performance, carcass characteristics, and bone mineralization of growing-finishing pigs fed increasing analyzed calcium to analyzed phosphorus (Ca:P) ratio from 26- to 127-kg body weight (BW) in Exp. 1[1]

Analyzed Ca:P ratio[2]:	0.75:1	1.00:1	1.25:1	1.50:1	1.75:1	2.00:1		Probability, *P* =	
Item[3] STTD Ca:STTD P[4]:	0.96:1	1.30:1	1.65:1	1.98:1	2.32:1	2.67:1	SEM	Linear	Quadratic
Grower period (d 0 to 56)									
ADG, g	902	930	927	910	943	923	11.1	0.129	0.279
ADFI, g	1,913	1,911	1,934	1,906	1,949	1,961	31.9	0.073	0.439
G:F, g/kg	472	487	480	478	485	471	5.0	0.791	0.037
Finisher period (d 56 to 110)									
ADG, g	942	977	994	985	951	932	18.6	0.390	0.009
ADFI, g	2,811	2,979	2,970	2,941	2,860	2,866	37.1	0.708	0.002
G:F, g/kg	335	328	335	3335	333	325	5.4	0.388	0.400
Overall period (d 0 to 110)									
ADG, g	923	955	961	949	950	929	10.4	0.953	0.005
ADFI, g	2,338	2,408	2,419	2,396	2,382	2,383	28.8	0.483	0.028
G:F, g/kg	395	397	398	396	399	390	3.9	0.479	0.116
BW, kg									
d 0	26.3	26.3	26.3	26.3	26.3	26.3	0.71	0.967	0.860
d 56	77.1	78.6	78.6	77.6	79.2	78.1	1.14	0.262	0.203
d 110	124.9	129.0	130.0	128.2	127.9	125.6	1.72	0.906	0.006
Carcass characteristics[5]									
HCW, kg	93.0	95.9	96.2	94.6	94.4	92.7	1.18	0.298	0.003
Yield, %	74.5	74.4	74.0	73.8	73.8	73.8	0.33	0.066	0.471
Backfat, mm[6]	16.8	16.3	16.8	16.9	16.3	16.5	-[7]	0.584	0.823
Fat-free lean, %[6]	56.8	56.7	56.9	57.1	57.3	57.0	-[7]	0.236	0.832
Loin depth, mm[6]	68.4	69.0	68.8	70.9	69.7	68.3	-[7]	0.597	0.067
Bone characteristics[8]									
Ash, %[6,9]	61.2	61.5	62.4	62.3	62.4	62.5	0.19	<0.001	0.017

[1] A total of 1,134 pigs (PIC 359 × 1050, initially 26.3 kg) were used in a 110-d growth trial with 27 pigs per pen and 7 pens per treatment.

[2] Treatments were formulated to be adequate in standardized total tract digestible phosphorus (STTD P) within phases, which corresponded to 0.38, 0.32, 0.29, and 0.26 % STTD P for phases 1, 2, 3, and 4, respectively.

[3] ADG= average daily gain. ADFI= average daily feed intake. G:F= gain-to-feed ratio. HCW= hot carcass weight.

[4] Coefficients for standardized total tract digestibility (STTD) of P were obtained from NRC (2012), while values for STTD Ca in feed ingredients were obtained from Stein (2016). These represent a weighted average of the ratio between STTD Ca:STTD P across treatments for the four dietary phases.

[5] 907 pigs were transported to a commercial packing plant for processing and data collection (Swift and Company, Worthington, MN).

[6] Adjusted for HCW.

[7] SEM for backfat were 0.31, 030, 0.29, 0.29, and 0.29; SEM for fat free lean were 0.30, 0.29, 0.28, 0.28,0.28 and 0.28; SEM for loin depth were 0.84, 0.82, 0.81, 0.81,0.80, and 0.81 for 0.75:1, 1.00:1, 1.25:1, 1.50:1, 1.75:1, and 2.00:1 analyzed total Ca:P, respectively.

[8] 84 pigs (2 pigs/pen, 1 barrow/1gilt) visually assumed to represent the mean live weight of the pen were subsampled and shipped to a separate processing facility for bone collection (Natural Foods Holdings, Inc., Sioux Center, IA). A total of 84 third metacarpals were autoclaved for 1h. After cleaning, bones were placed in Soxhlets containing pretoleum ether for 7 d as a means of removing water and fat. They were then dried at 105^0C for 7 d, and then ashed at 600^0C for 24h.

[9] The two-way interaction and the effect of gender were tested and no evidence for significant effects were observed.

Table 3.7 Least square means for growth performance, carcass characteristics, and bone mineralization of growing-finishing pigs fed increasing analyzed calcium to analyzed phosphorus (Ca:P) ratio from 26- to 127-kg body weight (BW) in Exp. 2[1]

Analyzed Ca:P ratio[2,3]:	0.75:1	1.00:1	1.25:1	1.50:1	2.00:1		Probability, *P* =	
Item[4] STTD Ca:STTD P[5]:	0.95:1	1.18:1	1.40:1	1.62:1	2.07:1	SEM	Linear	Quadratic
Grower period (d 0 to 58)								
ADG, g	820	838	830	852	830	12.3	0.390	0.061
ADFI, g	1,695	1,701	1,694	1,742	1,706	37.7	0.349	0.289
G:F, g/kg	485	494	491	489	487	5.7	0.908	0.179
Finisher period (d 59 to 114)								
ADG, g	895	952	980	982	994	11.7	<0.001	0.001
ADFI, g	2,685	2,769	2,814	2,825	2,816	41.8	0.011	0.044
G:F, g/kg	334	344	345	348	354	5.7	0.002	0.229
Overall (d 0 to 114)								
ADG, g	861	898	905	918	913	8.6	<0.001	0.001
ADFI, g	2,173	2,220	2,228	2,269	2,245	37.1	0.025	0.090
G:F, g/kg	397	405	407	405	407	4.7	0.018	0.116
BW, kg								
d 0	25.3	25.3	25.4	25.3	25.3	0.93	0.924	0.766
d 58	73.6	74.5	74.3	75.4	74.2	1.55	0.427	0.096
d 114	121.8	127.3	127.8	128.7	127.3	1.80	0.001	<0.001
Carcass characteristics[6]								
HCW, kg	90.4	92.9	93.6	94.6	94.0	1.33	0.002	0.014
Yield, %	74.4	73.1	73.2	73.5	73.6	0.42	0.550	0.090
Backfat, mm[7]	16.1	16.0	16.5	16.2	16.2	-[8]	0.855	0.604
Fat-free lean, %[7]	57.4	57.5	57.2	57.2	57.3	-[8]	0.650	0.615
Loin depth, mm[7]	69.5	70.2	69.8	68.7	69.2	-[8]	0.406	0.984
Bone characteristics[9]								
Ash, %[7,10]	60.5	61.1	61.9	61.8	61.8	0.19	<0.001	0.001

[1] A total of 1,214 pigs (PIC 337 × 1050, initial pen average BW of 55.7 lb) were used in a 114-d growth trial with 27 pigs per pen and 9 pens per treatment.

[2] Treatments were formulated to be adequate in STTD P within phases, which corresponded to 0.38, 0.32, 0.29, and 0.26 % STTD P for phases 1, 2, 3, and 4, respectively.

[3] Phytase (Ronozyme Hiphos, DSM Nutritional Products, Parsippany, NJ) was added to the diets at 1,000 FYT/kg feed with assumed release values of 0.15% avP, 0.132% STTD P, 0.144% Total Ca, and 0.096% STTD Ca.

[4] ADG= average daily gain. ADFI= average daily feed intake. G:F= gain-to-feed ratio. HWC= hot carcass weight.

[5] Coefficients for standardized total tract digestibility (STTD) of P were obtained from NRC (2012), while values for STTD Ca in feed ingredients were obtained from Stein (2016). These represent a weighted average of the ratio between STTD Ca:STTD P across treatments for the four dietary phases

[6] 907 pigs were transported to a commercial packing plant for processing and data collection (Swift and Company, Worthington, MN).

[7] Adjusted for HCW.

[8] SEM for backfat were 0.38, 036, 0.37, and 0.38; SEM for fat-free lean were 0.25, 0.24, 0.25, 0.25, and 0.25; SEM for loin depth were 0.71, 0.68, 0.68, 0.68, and 0.70 for the 0.75:1, 1.00:1, 1.25:1, 1.50:1, and 2.00:1 analyzed Ca:P ratio treatments, respectively.

[9] 90 pigs (2 pigs/pen, 1 barrow/1gilt) visually assumed to represent the mean live weight of the pen were subsampled and shipped to a separate processing facility for bone collection (Natural Foods Holdings, Inc., Sioux Center, IA). A total of 90 third metacarpals were autoclaved for 1h. After cleaning, bones were placed in Soxhlets containing pretoleum ether for 7 d as a means of removing water and fat. They were then dried at 1050C for 7 d, and then ashed at 6000C for 24h.

[10] The two-way interaction was tested and no evidence for significant interaction was observed. There was a marginal significant gender effect ($P < 0.10$) on percentage bone ash, with barrows having greater bone mineralization than gilts (61.6 and 61.3% for barrows and gilts, respectively).

Table 3.8 Best fitting models and calcium (Ca) to phosphorus (P) ratio for maximum response for different variables in Exp. 1 and Exp. 2[1]

		Analyzed Ca:P		STTD Ca:STTD P[2]	
Item[3]	Best Fitting Model[4]	Maximum response	95% CI[5]	Maximum response	95% CI[5]
Exp. 1[6]					
ADG	QP	1.38:1	1.00:1, 1.75:1	1.82:1	1.30:1, 2.31:1
ADFI	QP	1.49:1	0.90:1,>2.00:1	1.97:1	1.30:1,>2.67:1
ADFI	BLL	---	---	1.30:1	0.93:1,1.67:1
G:F	QP	1.29:1	<0.75:1,>2.00:1	1.69:1	<0.96:1,>2.67:1
HCW	QP	1.25:1	0.86:1,1.72:1	1.64:1	1.07:1,2.31:1
Bone ash	QP	1.93:1	1.40:1,>2.00:1	2.57:1	1.85:1,>2.67:1
Exp. 2[7]					
ADG	QP	1.63:1	1.25:1,>2.00:1	1.75:1	1.40:1,>2.07:1
ADFI	LM	2.00:1	---	≥2.07:1	---
G:F	BLL	1.05:1	0.81:1,1.30:1	---	---
G:F	LM	---	---	≥2.07:1	---
HCW	QP	1.60:1	1.14:1,>2.00:1	1.71:1	1.28:1,>2.07:1
HCW	BLL	1.11:1	0.87:1,1.36:1	1.28:1	1.06:1,1.50:1
Bone ash	BLL	1.25:1	1.20:1,1.40:1	1.40:1	1.26:1,1.54:1

[1] A total of 1,134 and 1,214 pigs (PIC 359 × 1050, initially 26.3 and 25.3 kg) were used in a 110-d and 114-d growth trials with 27 pigs per pen and 7 and 9 pens per treatment in Exp. 1 and in Exp. 2, respectively.

[2] Coefficients for standardized total tract digestibility (STTD) of P were obtained from NRC (2012), while values for STTD Ca in feed ingredients were obtained from Stein et al. (2016). These represent a weighted average of the ratio between STTD Ca:STTD P across treatments for the four dietary phases.

[3] ADG= average daily gain. ADFI= average daily feed intake. G:F= gain-to-feed ratio. HWC= hot carcass weight.

[4] Results represent inferences yield based on the best fitting models. The best fitting models were selected based on the Bayesian Information Criteria (Miliken and Johnson, 2009). The competing models included a linear (LM), quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ).

[5] CI = confidence interval.

[6] Treatments were formulated to 0.75:1, 1.00:1, 1.25:1, 1.50:1, 1.75:1, and 2.00:1 analyzed calcium to analyzed phosphorus ratios across dietary phases. These represent a weighted average of 0.96:1, 1.30:1, 1.65:1, 1.98:1, 2.32:1, and 2.67:1 STTD Ca:STTD P ratios. Treatments were achieved with the addition of limestone at the expense of corn.

[7] Treatments were formulated to 0.75:1, 1.00:1, 1.25:1, 1.50:1, and 2.00:1 analyzed calcium to analyzed phosphorus ratios across dietary phases. These represent a weighted average of 0.95:1, 1.18:1, 1.40:1, 162:1and 2.07:1 STTD Ca:STTD P ratios. Treatments were achieved with the addition of limestone at the expense of corn. Phytase (Ronozyme HiPhos, DSM Nutritional Products, Parsippany, NJ) was included at 1,000 FYT/kg releasing an assumed 0.15% aP, 0.132% STTD P, 0.144% total Ca, and 0.096% STTD Ca.

Table 3.9 Equations based on the best fitting models for different response criteria in Exp. 1 and Exp. 2[1,2,3]

Item[4]	Equations
Exp. 1 Analyzed Ca:P	
ADG, g	= 806.20 + 222.10 × (analyzed Ca:P) – 80.5513 × (analyzed Ca:P)^2
ADFI, g	= 2219.21 + 245.73 × (analyzed Ca:P) – 82.6743 × (analyzed Ca:P)^2
G:F, g/kg	= 376.77 + 33.5039 × (analyzed Ca:P) – 13.0113 × (analyzed Ca:P)^2.
HCW, kg	= 87.87 + 11.359 × (analyzed Ca:P) – 4.542 × (analyzed Ca:P)^2
Bone ash, %	= 58.91 + 3.67229 × (analyzed Ca:P) – 0.95170 × (analyzed Ca:P)^2
Exp. 1 STTD Ca:STTD P[5]	
ADG, g	= 817.31 + 155.98 × (STTD Ca:STTD P) – 42.8764 × (STTD Ca:STTD P)^2.
ADFI, g	= 2231.27 + 173.21 × (STTD Ca:STTD P) – 44.0058 × (STTD Ca:STTD P)^2.
ADFI, g	= 2392.79 – 161.07 × (1.2988 – STTD Ca:STTD P) if STTD Ca:STTD P ratio < 1.30:1 = 2392.79 if STTD Ca:STTD P ratio ≥ 1.30:1
G:F, g/kg	= 378.33 + 23.6212 × (STTD Ca:STTD P) – 6.9747 × (STTD Ca:STTD P)^2.
HCW, kg	= 194.96 + 17.5016 × (STTD Ca:STTD P) – 5.3251 × (STTD Ca:STTD P)^2.
Bone ash, %	= 59.068 + 2.63163 × (STTD Ca:STTD P) – 0.51217 × (STTD Ca:STTD P)^2.
Exp. 2 Analyzed Ca:P	
ADG, g	= 729.12 + 233.25 × (analyzed Ca:P) –71.3315 × (analyzed Ca:P)^2
ADFI, g	= 2109.55 + 98.8323 × (analyzed Ca:P)
G:F, g/kg	= 406.43 - 31.4661 × (1.0543 – analyzed Ca:P) if analyzed Ca:P ratio < 1.05:1 = 406.43 if analyzed Ca:P ratio ≥ 1.05:1
HCW, kg	= 80.862 + 17.112 × (analyzed Ca:P) – 5.351 × (analyzed Ca:P)^2
HCW, kg	= 94.03 – 9.622 × (1.11 – analyzed Ca:P) if analyzed Ca:P ratio < 1.11:1 = 94.03 if analyzed Ca:P ratio ≥ 1.11:1
Bone ash, %	= 61.83 – 2.65158 × (1.25 - analyzed Ca:P) if analyzed Ca:P ratio < 1.25:1 = 61.83 if analyzed Ca:P ratio ≥ 1.25:1
Exp. 2 STTD Ca:STTD P	
ADG, g	= 650.05 + 309.12 × (STTD Ca:STTD P) –88.5666 × (STTD Ca:STTD P)^2
ADFI, g	= 2068.89 + 114.64 × (STTD Ca:STTD P)

G:F, g/kg	= 393.25 + 7.5673 × (STTD Ca:STTD P)
HCW, kg	= 75.051 + 22.761 × (STTD Ca:STTD P) – 6.646 × (STTD Ca:STTD P)^2.
HCW, kg	= 94.03 – 10.574 × (1.2787 – STTD Ca:STTD P) if STTD Ca:STTD P ratio < 1.28:1
	= 94.03 if STTD Ca:STTD P ratio ≥ 1.28:1
Bone ash, %	= 61.827 – 2.94931 × (1.40 – STTD Ca:STTD P) if STTD Ca:STTD P ratio < 1.40:1
	= 61.827 if STTD Ca:STTD P ratio ≥ 1.40:1

[1] A total of 1,134 and 1,214 pigs (PIC 359 × 1050, initially 26.3 and 25.3 kg) were used in a 110-d and 114-d growth trials with 27 pigs per pen and 7 and 9 pens per treatment in Exp. 1 and in Exp. 2, respectively.

[2] Treatments were formulated to 0.75:1, 1.00:1, 1.25:1, 1.50:1, 1.75:1, and 2.00:1 analyzed calcium to analyzed phosphorus ratios across dietary phases. These represent a weighted average of 0.96:1, 1.30:1, 1.65:1, 1.98:1, 2.32:1, and 2.67:1 STTD Ca:STTD P ratios. Treatments were achieved with the addition of limestone at the expense of corn.

[3] Treatments were formulated to 0.75:1, 1.00:1, 1.25:1, 1.50:1, and 2.00:1 analyzed calcium to analyzed phosphorus ratios across dietary phases. These represent a weighted average of 0.95:1, 1.18:1, 1.40:1, 162:1and 2.07:1 STTD Ca:STTD P ratios. Treatments were achieved with the addition of limestone at the expense of corn. Phytase (Ronozyme HiPhos, DSM Nutritional Products, Parsippany, NJ) was included at 1,000 FYT/kg releasing an assumed 0.15% aP, 0.132% STTD P, 0.144% total Ca, and 0.096% STTD Ca.

[4] Ca= calcium. P= phosphorus. ADG= average daily gain. ADFI= average daily feed intake. G:F= gain-to-feed ratio. HWC= hot carcass weight.

[5] Coefficients for standardized total tract digestibility (STTD) of P were obtained from NRC (2012), while values for STTD Ca in feed ingredients were obtained from Stein et al. (2016). These represent a weighted average of the ratio between STTD Ca:STTD P across treatments for the four dietary phases.

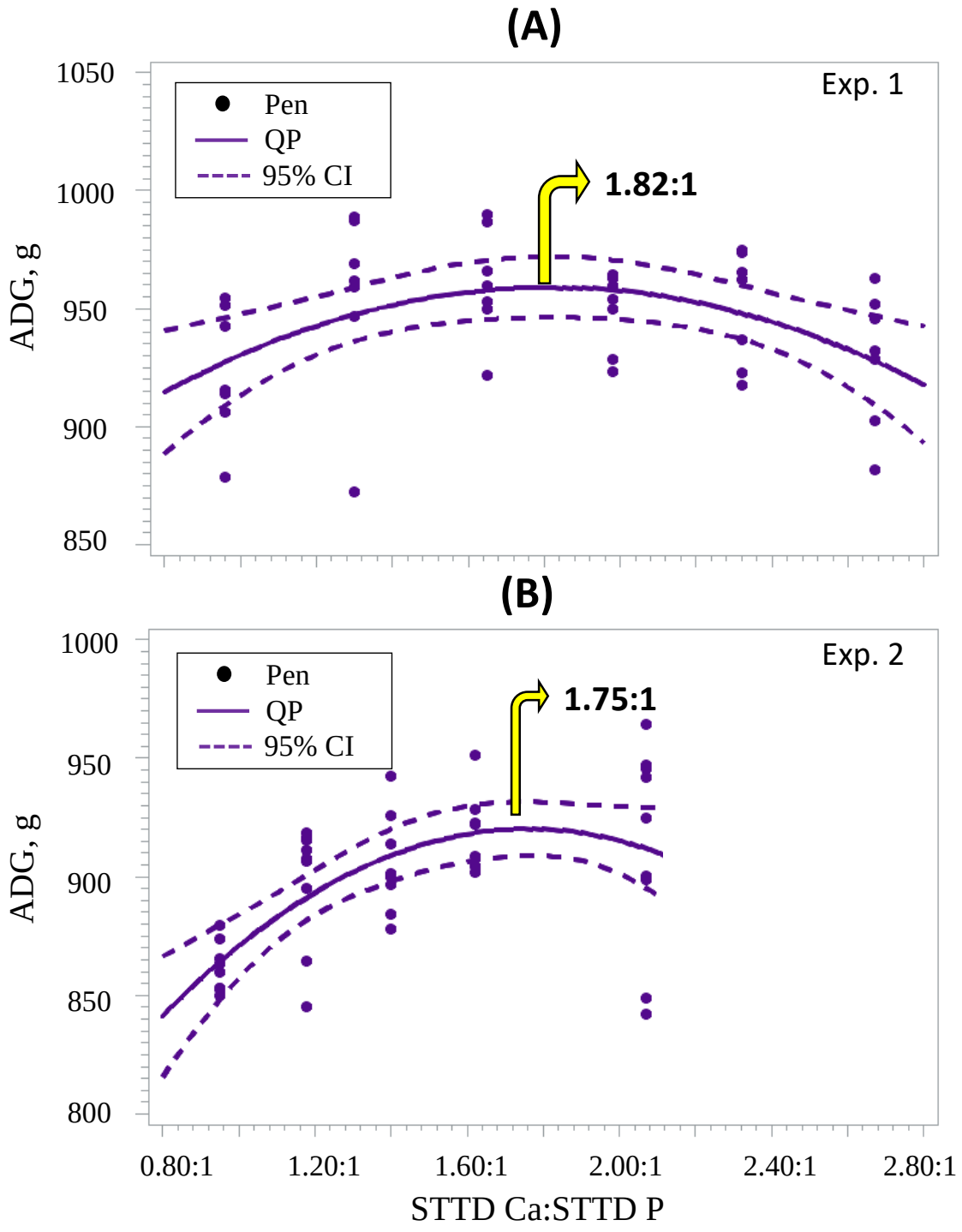

Figure 3.1 Fitted quadratic polynomial (QP) regression model on average daily gain (ADG) as a function of increasing standardized total tract digestible (STTD) Ca:STTD P ratio in growing-finishing pigs. (A) In Exp. 1, the QP model estimated the maximum mean ADG at 1.82:1 STTD

Ca:STTD P ratio (95% CI: [1.30:1,2.31:1]). The estimated regression equation was ADG, g = 817.31 + 155.98 × (STTD Ca:STTD P) – 42.8764 × (STTD Ca:STTD P)^2. (B) In Exp. 2, the QP model estimated the maximum mean ADG at 1.75:1 STTD Ca:STTD P ratio (95% CI: [1.40:1, >2.07:1]). Based on the best fitting model, the estimated regression equation was ADG, g = 650.05 + 309.12 × (STTD Ca:STTD P) –88.5666 × (STTD Ca:STTD P)^2

Chapter 4 - Economic model for optimum standardized total tract digestible phosphorus for finishing pigs

ABSTRACT

An adequate supply of dietary phosphorus (P) is important for pig growth performance and bone mineralization. However, P represents the third most expensive nutrient in swine diets after energy and protein, and can greatly affect diet cost. Therefore, the objective of this project was to develop a tool to compare current dietary standardized total tract digestible (STTD) P concentrations to suggested values that yield maximum growth performance while accounting for different financial scenarios. The P economic tool is a Microsoft Excel®-based model that evaluates the user's current dietary STTD P concentrations for a specific production system and market conditions. The tool takes into consideration whether the system is marketing pigs on a fixed time or fixed weight basis. Moreover, the user has the option of an imperial or metric version, as well as the evaluation using two different energy systems: metabolizable energy and net energy. Data from Vier et al. (2017, 2019a) have described the dose response curve to increasing STTD P for late nursery and finishing pigs under commercial conditions. Based on these data, regression equations were developed to predict the STTD P requirement, as a percentage of the diet, for maximum growth rate according to the energy content of the user's diets. For model calculations, non-linear regression equations for average daily gain and feed efficiency are used. The tool calculates profitability indicators utilizing a live or carcass weights. For profitability calculations on a carcass basis, a regression equation was developed to account for the effect of STTD P on carcass yield. This tool provides a means for the users to compare their current STTD P concentrations to levels required to achieve maximum growth performance, while considering the financial implications under dynamic productive and

economic situations. The model can be accessed at www.ksuswine.org. or at the open science framework data repository (Vier, 2019b).

Key words: digestible phosphorus, economic tool, growth, finishing pigs

INTRODUCTION

An adequate supply of dietary phosphorus (**P**) is important for pig growth performance and bone mineralization. It is well established that after the skeleton, the greatest body reserve of P is the muscle tissue, with minimum P found in the adipose tissue (Nielsen, 1973). Moreover, the greater the ratio of lean tissue growth, the greater the demand for P to support this growth (Jongbloed, 1987). Therefore, genetic improvements towards increased pig performance and lean tissue growth over time may result in greater P requirements than in the past. In fact, a recent study conducted in a commercial setting has demonstrated that the standardized total tract digestible (**STTD**) P requirement of modern genotype is greater than NRC (2012) estimates on a dietary percentage basis (Vier et al., 2017). This study resulted in the development of non-linear regression equations to predict growth rate, feed efficiency, and carcass yield according to dietary STTD P concentration.

It is important to note that after energy and protein, P represents the third most expensive nutrient in swine diets (Fan et al., 2001). Therefore, P concentration can impact dietary cost. Also due to the nonlinear nature of the response, the dietary STTD P to support maximal growth will not always result in maximal profitability. The objective of this study was to develop a tool to compare current dietary STTD P concentrations to recommended values that yield maximum growth performance while accounting for financial implications over different scenarios.

MATERIALS AND METHODS

Model Description

The phosphorus economic tool is a Microsoft Excel®-based model and is intended to be used by swine nutritionists. This tool provides a method to evaluate current dietary STTD P concentrations for a specific production system and market conditions. The tool takes into consideration whether the system is marketing pigs on a fixed time (where increased gain is important) or fixed weight basis (where gain is not valued because days are adequate to reach the desired market weight). Moreover, the user has the option of an imperial or metric version, as well as two different energy systems: metabolizable energy (**ME**) or net energy (**NE**). The P economic tool is divided into 3 sections: 1) user inputs, with economic and dietary criteria; 2) background model calculations for growth performance, carcass yield predictions, and profitability indexes; and 3) model outputs with recommended STTD P concentration for maximal growth, percentage of maximal growth performance for the current STTD P concentrations, and profitability indexes that contrast the current and estimated STTD P for maximal growth.

User Inputs

The user has the option to choose either the ME or NE basis according to the energy system used in the production system. Once the energy basis is defined, the user is required to enter the following inputs for calculation of growth performance and economic criteria: pork carcass price ($/lb or $/kg), facility cost ($/pig/d), and the current carcass yield (%). In addition, the user is required to select the economic evaluation criteria (live or carcass basis) and the number of dietary phases (the model allows the selection of 2 to 6 phases).

After defining the number of dietary phases, the user is required to enter the body weight (**BW**) ranges within each phase, along with the energy concentration of each diet (kcal/lb or kcal/kg). Then, the user enters the current dietary STTD P (%) concentrations for each dietary phase and the associated diet costs. The model will then calculate the STTD P concentration to achieve maximum growth based on the BW ranges and the specified energy content of the diets. The user is required to reformulate their diets with the STTD P concentrations suggested by the model and subsequently input the associated dietary costs. This step is required for the economic comparisons between the current STTD P levels provided by the user and the model recommended STTD P levels for maximum growth.

Calculations for Performance and Economics

Energy content of the diet can affect feed intake, therefore, this model calculates the STTD P estimates as a ratio relative to energy. Data from Vier et al. (2017, 2019) have described the dose response curve to increasing STTD P for late nursery and finishing pigs under commercial conditions. Based on these data, two sets of equations were developed to estimate the STTD P to energy ratio as a function of BW:

$$\text{STTD P:NE, g/Mcal} = 0.0000472912571538526 \times (\text{BW, kg})^2 - 0.0143907820290028 \times (\text{BW, kg}) + 2.0275145422229$$

$$\text{STTD P:ME, g/Mcal} = 0.0000306269361758696 \times (\text{BW, kg})^2 - 0.00966436147205444 \times (\text{BW, kg}) + 1.47675067863161$$

The equation result is then multiplied by the input provided by the user (energy content of the diet) and converted from g/kg to predict the STTD P requirement, as a percentage of the diet, for maximum growth.

This model also utilizes average daily gain (**ADG**) and gain-to-feed (**G:F**) predicted equations developed by Vier et al.(2017):

ADG, g = 651.36 + 531.33 × (STTD P as % of NRC) – 216.90 × (STTD P as % of NRC)^2.

G:F, g/kg = 338.34 + 108.98 × (STTD P as % of NRC) – 46.7864 × (STTD P as % of NRC)^2.

The phase duration is determined based on the initial and final BW and the calculated ADG (Table 1). The equations to predict ADG and G:F described above were developed based on the overall finisher performance. Therefore, to calculate feed intake within each dietary phase, first a weighted average of the feed efficiency based on the phase duration is determined. This overall feed efficiency is then used with the KSU Feed Budget Calculator (access at ksuswine.org) to obtain the feed intake per dietary phase. In the fixed time scenario, the predicted final BW is included in the feed budget calculator to account for the extra feed intake.

Data developed from a reference population (PIC 337 growing finishing pigs; provided by U. Orlando Genus PIC) is used to calculate the predicted carcass yield as influenced by changes in body weight. The predicted carcass yield is then adjusted based on the current carcass yield provided by the user. Furthermore, data from Vier et al. (2017) suggested that carcass yield decreases as the concentration of STTD P increases. The estimated regression equation to predict carcass yield according to STTD P concentration is as follow:

Carcass yield, % = 73.859 – 1.19192 × (STTD P as % of NRC)

Therefore, the predicted carcass yield is adjusted based on a weighted average of the STTD P concentrations within dietary phases compared to a reference carcass yield (yield at 100 % of NRC STTD P estimates).

Fixed weight scenario calculations on a carcass basis are based on the user's predicted HCW. Due to the negative impact of STTD P on carcass yield, pigs would have to be fed to a heavier final BW in the fixed weight carcass basis situation to achieve a carcass weight similar to the user's input. Economic variables are then calculated based on the sum of costs across phases (Table 1).

RESULTS AND DISCUSSION

Application of the Model

Two examples using this model are presented in Tables 2 and 3. In both examples, a six-phase feeding program (25 to 34, 34 to 50, 50 to 64, 64 to 84, 84 to 107, and 107 to 129 kg) was used. In example 1, diets were corn-soybean meal based, and the STTD P levels were achieved with monocalcium phosphate and added phytase. In the second example, the diets were corn-soybean meal- distillers dried grains with solubles (**DDGS**) based with added phytase. Phases 1 to 4 had the inclusion of 25% DDGS, which was reduced to 22.5 and 10.0% in phases 5 and 6.

In both simulations, diets were formulated similar to NRC STTD P estimates across dietary phases. Therefore, diets contained 0.32, 0.30, 0.28, 0.26, 0.23, and 0.21% STTD P in example 1. Diets in example 2 contained 0.33, 0.30, 0.27, 0.26, 0.23, and 0.21% STTD P. The NE system was used, with diets containing 2,454, 2,480, 2.509, 2,538, 2,564 and 2,573 kcal NE/kg of diet in example 1, and 2,425, 2,449, 2,482, 2,509, 2,549 and 2,564 kcal NE/kg of diet in example 2. The model estimated the STTD P concentration for maximal growth at 0.40, 0.37, 0.34, 0.31, 0.28, and 0.25% for phases 1 to 6, respectively.

For scenario building in example 1, the following inputs were used: 1) facility cost of \$0.12/pig/d; 2) current carcass yield of 73.4%; 3) current diet costs of \$181.93, \$175.17, \$167.76, \$160.81, \$156.04 and \$153.56 per ton; and 4) diet costs of reformulated diets to STTD

P for maximal growth of $183.29, $176.36, $168.87, $161.75, $156.89 and $154.18 per ton. For scenario building in example 2, the following inputs were used: 1) facility cost of $0.12/pig/d; 2) current carcass yield of 73.4%; 3) current diet costs of $173.65, $166.98, $160.96, $155.51, $153.23 and $152.78 per ton; and 4) diet costs of reformulated diets to STTD P for maximal growth of $174.03, $167.22, $161.15, $155.65, $153.32 and $152.92 per ton.

For calculation of feed costs presented above, the pricing of main ingredients used were: corn ($0.139/kg), soybean meal ($0.295/kg), DDGS ($0.132/kg), monocalcium phosphate ($0.498/kg), and phytase ($2.205/kg). To evaluate the model performance, carcass value was modified from moderate ($1.43/kg) to high ($1.81/kg) market prices.

Scenario Results

Approximately 98.9 and 99.7% of the maximum ADG and G:F can be captured using the current dietary STTD P concentrations in both examples. The economics at these STTD P concentrations were calculated and compared with the economics at STTD P concentrations needed to achieve maximum growth.

In example 1, increasing STTD P above current levels resulted in an increase in total feed cost and total feed and facility cost both in a fixed weight and fixed time basis. Revenue per pig was the same on a fixed weight basis. On a fixed time scenario, due to improvements in growth performance, pigs fed increased STTD P reached a greater final BW compared to pigs fed diets with the current STTD P levels. Therefore, revenue per pig increased on a fixed time basis even with the negative impact of increasing STTD P on carcass yield.

Regardless of a moderate or high carcass price, it was not economical to increase the STTD P concentration to achieve maximal growth when the system is working on a space long situation. Increasing STTD P above current levels resulted in a reduction in income over feed

cost (**IOFC**) of $0.40 and income over feed and facility costs (**IOFFC**) of $0.31/pig. If the system is working on a space short situation, it is economical to increase the STTD P levels. The IOFC and IOFFC were $0.11 and $0.30/pig higher in a moderate and high carcass price situation, respectively.

In example 2, increasing STTD P above current levels resulted in an increase in total feed cost and total feed and facility cost on a fixed time and fixed weight basis. Similar to example 1, revenue per pig was similar between the current diets and diets for maximum growth as there was time to raise pigs to the same desired carcass weight. Regardless of a moderate or high carcass price, IOFC and IOFC was decreased with increasing STTD P when the system is on a space long situation. However, when the system is marketing on a fixed time, or on a space short situation, increasing STTD P in diets containing corn, SBM, DDGS, and phytase resulted in improvements of $0.36/pig in IOFC and IOFFC in a scenatio with moderate carcass price. Considering a scenario with high carcass price, increasing the STTD P resulted in an improvement of $0.57/pig in IOFC and IOFFC.

As illustrated, a key concept is understanding if pigs are marketed on a fixed time or fixed weight basis. A greater response to increasing STTD P is observed for growth rate compared to feed efficiency, and the fixed time or fixed weight situations change the relative value of the growth rate. Most pig production systems fluctuate between a fixed time and fixed weight scenario based on pig flow, growth seasonality, and pig space availability. Due to ingredient price and differences in formulation, profit per pig was greater in example 2 compared to example 1 on a fixed time basis. However, it is worth noting that increasing STTD P above current levels to a concentration needed to achieve maximum growth in a fixed time basis increased the income per pig in both examples. Due to the fixed constraint on the number of days

available for growth, the growth rate value is greater in a fixed time scenario. However, in most fixed weight scenarios where adequate growing space is available, it will not be economical to increase the STTD P above the suggested NRC dietary requirement. In this situation, pigs can stay in the barn at a fixed space cost per day until they reach the desired market weight. Therefore, the value of faster weight gain is lower than if on a fixed-time basis.

Model Limitations

Currently, the model only estimates performance and economics according to STTD P levels for mixed gender pigs. The BW range used to develop the regression equations used in this model is 56- to 285-lb (26- to 130-kg). Model predictions outside this BW range are not recommended and should be used with caution. In addition, the model does not predict the STTD P level that yields the greatest profitability. It only compares the economics between the current STTD P levels and the STTD P levels needed for maximum growth.

Summary

The model described herein is intended to be used by swine nutritionists. This tool provides a method to evaluate current dietary STTD P concentrations for a specific production system and market conditions. It can be used to compare current dietary STTD P concentrations to recommended values that yield maximum growth performance while accounting for financial implications over different scenarios. To evaluate the performance of the model, two examples are presented considering different dietary formulations and different economic scenarios created by modifying carcass pricing in a fixed time and fixed weight situations.

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Table 4.1 Input equations used in model development.

Indicator[1]	Calculation
Phase duration[2,3], d (Fixed weight live basis)	= [(Final BW, lb – Initial BW, lb)/2.2046]/(calculated ADG, g / 1000)
Gain per phase, lb	= (Calculated ADG, g/1000) × (Phase duration, d) × 2.2046
Calculated HCW[4], lb	= (∑ gain per phase, lb + Initial BW, lb) × (Predicted carcass yield, %/100)
Feed cost per phase, $/pig	= Feed budget by phase, lb/pig × (diet cost, $/ton)/2000
Feed and facility cost per phase, $/pig	= (Feed cost per phase, $/pig) + (Phase duration, d × facility cost, $/pig/d)
Revenue per pig, $/pig (live basis)	= (∑ gain per phase, lb + Initial BW, lb) × Live price, $/lb
Revenue per pig, $/pig (carcass basis)	= (Calculated HCW, lb) × (Carcass price, $/lb)
Income over feed cost, $/pig	= (Revenue per pig, $/pig) – (∑ feed cost per phase, $/pig)
Income over feed and facility cost, $/pig	= (Revenue per pig, $/pig) – (∑ feed and facility cost per phase, $/pig)

[1]BW = body weight. ADG = average daily gain. HCW = hot carcass weight.

[2]Calculation of phase duration for fixed time is based on user predicted duration in each phase.

[3]Final BW for fixed weight carcass basis = (Calculated HCW for current performance, lb) × (Predicted carcass yield,%/100)

[4]Calculation of HCW for fixed weight is based on user predicted HCW.

Table 4.2 Overall growth performance and economics of user defined STTD P levels compared with model recommended STTD P levels for maximal growth in a six-phase feeding program with varying pig carcass pricing on a fixed time and fixed weight marketing basis: Example 1[1]

	Carcass price, $/kg					
	1.43			1.81		
		Maximum growth			Maximum growth	
Item[2]	Current	Fixed weight	Fixed time	Current	Fixed weight	Fixed time
Growth performance, % of maximum						
ADG	98.9	100	100	98.9	100	100
F/G	99.7	100	100	99.7	100	100
Economics, $/pig						
Total feed cost	46.42	46.82	47.04	46.42	46.82	47.04
Total feed and facility cost	59.39	59.70	60.01	59.39	59.70	60.01
Total revenue	135.71	135.71	136.43	171.78	171.78	172.69
IOFC	89.29	88.90	89.40	125.36	124.96	125.65
IOFFC	76.32	76.01	76.43	112.38	112.07	112.68

[1] Example 1 consisted of a six-phase feeding program (25 to 34, 34 to 50, 50 to 64, 64 to 84, 84 to 107, and 107 to 129 kg) with corn-soybean meal based diets that contained the inclusion of monocalcium phosphate and phytase. Price of ingredients were: corn ($0.139/kg), soybean meal ($0.295/kg), monocalcium phosphate ($0.498/kg), and phytase ($2.205/kg).

[2] ADG = average daily gain. F/G = feed-to-gain ratio. IOFC = income over feed cost. IOFFC = income over feed and facility cost.

Table 4.3 Overall growth performance and economics of user defined STTD P levels compared with model recommended STTD P levels for maximal growth in a four-phase feeding program with varying pig carcass pricing on a fixed time and fixed weight marketing basis: Example 2[1]

	Carcass price, $/kg					
	1.43			1.81		
		Maximal growth			Maximal growth	
Item[2]	Current	Fixed weight	Fixed time	Current	Fixed weight	Fixed time
Growth performance, % of maximum						
ADG	98.9	100	100	98.9	100	100
F/G	99.7	100	100	99.7	100	100
Economics, $/pig						
Total feed cost	45.23	45.39	45.63	45.23	45.39	45.63
Total feed and facility cost	58.20	58.27	58.61	58.20	58.27	58.61
Total revenue	135.72	135.72	136.49	171.78	171.78	172.75
IOFC	90.49	90.33	90.85	126.56	126.39	127.12
IOFFC	77.52	77.44	77.88	113.58	113.51	114.15

[1] Example 2 consisted of a six-phase feeding program (25 to 34, 34 to 50, 50 to 64, 64 to 84, 84 to 107, and 107 to 129 kg) with corn-soybean meal-DDGS based diets that contained the inclusion of phytase. Price of ingredients were: corn ($0.139/kg), soybean meal ($0.295/kg), distillers dried grains with solubles ($0.132/kg), and phytase ($2.205/kg).

[2] ADG = average daily gain. F/G = feed-to-gain ratio. IOFC = income over feed cost. IOFFC = income over feed and facility cost.

Chapter 5 - Stability of commercial phytase sources stored under high temperature and humidity and the effects on performance of nursery pigs

ABSTRACT

A study was conducted to evaluate the effects of storing three commercially available phytase products over 90 d under high temperature and high humidity conditions on phytase stability, growth performance, bone mineralization, and serum myo-inositol concentration of nursery pigs. The phytase sources [HiPhos GT (20,000 FYT/g, DSM Nutritional Products, Parsippany, NJ); Axtra Phy TPT (20,000 FTU/g, Dupont, Wilmington, DE), and Quantum Blue G (40,000 FTU/g, AB Vista, Plantation, FL)] were left as pure forms or blended in a vitamin and trace mineral premix (VTM) for a 90 d period in an environmentally controlled chamber set at 29.4°C and 75% humidity. Sampling occurred on d 0, 30, 60, and 90 of storage. There was no interaction between storage time, source, and form ($P > 0.05$). Phytase activity decreased (linear, $P < 0.05$) when storage time increased and when phytases were stored in VTM compared to pure form ($P < 0.05$). Then, a total of 300 nursery pigs (DNA 241 × 600; Columbus, NE, initially 11.7 kg) were used in a 21-d experiment, with 4 to 5 pigs per pen and 8 replicate pens per treatment. Pigs were fed a common diet with 0.12% available phosphorus (aP) for 4 d prior to the trial. Pens of pigs were randomly assigned to 1 of 8 dietary treatments in a randomized complete block design, with body weight blocks. Experimental diets were formulated to contain 0.12% aP (negative control, NC) or 0.27% aP (positive control, PC) supplied by an inorganic P; or the 0.12% aP diet with added phytase to provide the activity recommended by the manufacturer of

each phytase source to release 0.15% aP. These diets were manufactured with each phytase source previously stored either in a pure form or in a VTM for 90 d. Pigs fed PC had greater ($P < 0.05$) average daily gain compared to pigs fed Axtra Phy in VTM or NC. Feed intake was decreased for pigs fed NC ($P < 0.05$) compared to the other treatments, with Axtra Phy stored in VTM intermediate. Pigs fed PC or HiPhos stored in pure form had improved ($P < 0.05$) feed efficiency compared to pigs fed NC. Bone mineralization was greater ($P < 0.05$) for pigs fed PC compared to the other treatments, with HiPhos stored in pure form intermediate. No evidence of differences was observed for serum myo-inositol concentration. In conclusion, residual phytase activity decreased when phytases were stored in VTM compared to pure form. Except for Hiphos in pure form, bone ash was reduced when phytases were stored for 90 d compared to the PC.

Key words: growth, nursery pigs, modeling, phosphorus requirement, phytase, stability

INTRODUCTION

Microbial phytases are commonly used in swine diets to reduce the antinutritional effect of phytate by cleaving the phytate bound phosphorus (**P**) found in most feedstuffs of plant origin. As a consequence, the amount of P available to the pig is increased while the impact of P excretion in the environment is decreased (Selle and Ravindran, 2008). However, as with any catalytic proteins, phytases can easily and irreversibly lose activity when exposed to heat, moisture, and mechanical pressure from pelleting (Jongbloed and Kemme, 1990, Ward, 2002, Iyer and Ananthanarayan, 2008).

In addition to the manufacturing process, previous research has demonstrated that the storage conditions can also affect efficacy of phytase (Sulabo et al., 2011). These authors have shown that storing phytase in a pure form in ambient temperatures greater than 23°C with high humidity is detrimental to the stability of this enzyme. Moreover, the authors also reported

increased phytase degradation as the duration of storage increased up to 360 d due to potential interactions with components of vitamin and trace mineral (**VTM**) premixes. Similarly, De Jong et al. (2016) also observed a reduction in phytase activity when the enzyme was stored blended in a VTM premix compared to storage in a pure form or blended in a vitamin premix.

The activity of phytase is typically measured and determined at the time it is manufactured, not at the time it is used. However, the fundamental benefit of a phytase product relies on its ability to improve P availability to the pig at the time of use, thus depending on its stability. Therefore, the objective of the present study was to determine the effects of a 90 d storage period under high temperature and high humidity conditions for three commercially available phytase products stored in pure form or in a VTM premix on phytase stability, and then on growth performance, bone mineralization, and serum myo-inositol concentration of nursery pigs.

MATERIALS AND METHODS

General

The storage part of this study was conducted at the Bioprocessing and Industrial Value Added Program Building at Kansas State University, and the growth part of this study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. The Kansas State University Institutional Animal Care and Use Committee (Manhattan, KS) approved all experimental procedures in this study.

Phytase Sources

Three commercially available phytases were used in this study: HiPhos GT (coated, manufacturer declared concentration of 20,000 phytase units (FYT)/g; DSM Nutritional Products, Parsippany, NJ), Axtra Phy TPT (coated, minimum declared concentration of 20,000

phytase units (FTU)/g; Dupont, Wilmington, DE), and Quantum Blue G (uncoated but marketed as heat stable, minimum declared concentration of 40,000 FTU/g; AB Vista, Plantation, FL). The phytase products were obtained through a third-party distributor.

Phytase Stability

The three phytase sources were obtained and either stored for 90 d in an environmentally controlled chamber set at 29.4°C and 75% humidity as the straight product or used to create a VTM that was then stored. The amount of each phytase product added to the VTM was determined such that including 0.15% VTM premix in a diet would provide the activity of phytase recommended by the manufacturer to release 0.15% available P (**aP**; 1,000 FYT/kg feed for HiPhos, 651 FTU/kg feed for Axtra Phy, and 500 FTU/kg feed for Quantum Blue). Each phytase product was added to a concentrated phytase-free VTM premix (Table 1) on d 0 of storage to create 9.1 kg batches by mixing for 5 min in a paddle mixer. The phytase-free VTM premix, the three pure phytase products, and the three batches of VTM premix with each phytase source were bagged into single-lined paper bags for storage. On d 90 of storage, the phytase sources stored in pure form were then added to the phytase-free VTM premix. The amount of each phytase product added to the VTM on d 90 was the same as the amount of each phytase added to the VTM on d 0 of storage. Subsequently, the VTM premixes containing the phytase sources stored for 90 d in pure form or in the VTM were added to a corn-SBM diet and used in a growth study.

During storage, six samples from each bag were taken on d 0, 30, 60 and 90, except for the phytase-free VTM premix, which was only sampled on d 90. Before sampling, each bag was mixed to ensure that a representative sample was collected. Each sample of pure phytase products weighed approximately 50 g, and each sample of VTM premix with the phytases

weighed approximately 100 g. Immediately after collection, samples were sent to the laboratory for analysis (Technical Marketing Analytical Services, DSM Nutritional Products Inc., Belvidere, NJ). Each sample was assigned with a code and sent to the laboratory, so the sources were blinded. Procedures for analysis used a slight modification of AOAC (2000) official method 2000.12 (Engelen et al., 1994, 2001). Results were sent back to K-State with the assigned codes, which were then linked to the product source for statistical analysis.

Animals and Diets

After the 90-d storage period, the phytase-free VTM premix, the three pure phytase products, and the three VTM premixes with each phytase were used as part of a growth study. One room with completely slatted flooring and a deep pit for manure storage was used. Each pen (1.5 × 1.2 m) was equipped with a 4-hole dry self-feeder and a nipple waterer to provide *ad libitum* access to feed and water. A total of 300 nursery pigs (Line 241 × 600; DNA, Columbus, NE) were used in a 21-d trial. Pigs were weaned at approximately 21 d of age and allotted to pens of 4 or 5 pigs according to initial body weight (**BW**) and gender upon entry in the nursery. At weaning, pigs were fed a common pelleted phase 1 diet and a common meal phase 2 diet for 21 d. Four days before the initiation of the trial, all pigs were fed a common diet deficient in phosphorus (0.12% aP). On d 0 of the trial (initial average BW of 11.7 kg), the pens of pigs were randomly assigned to 1 of 8 dietary treatments in a randomized complete block design. There were 8 replicate pens per treatment and BW was used as the blocking factor.

All experimental diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center and fed in meal form. The eight experimental treatments consisted of: a negative control (NC), a positive control (PC), or the NC plus HiPhos, Axtra Phy, or Quantum Blue stored for 90 d in pure form, and HiPhos, Axtra Phy, or Quantum Blue stored

for 90 d in a VTM premix. The NC and PC diets were formulated with the addition of 0.15% phytase-free VTM premix. They were formulated to 0.12 and 0.27% aP, respectively, achieved with the inclusion of inorganic P provided by monocalcium phosphate. The remaining treatments were formulated to 0.27% aP, which were achieved with 0.15% aP released from each phytase product in addition to the 0.12% from the basal diet.

A total of 3 samples of corn, soybean meal, and monocalcium phosphate used in the diets were analyzed for P in duplicate (method 985.01; AOAC International, 1990; Ward Laboratories, Inc., Kearney, NE). The average of the six lab results for each ingredient was used for diet formulation (0.31, 0.66, and 20.54% P in corn, soybean meal, and monocalcium phosphate, respectively). Eight, 1-ton batches of basal diet were manufactured and bagged (Table 2). For each experimental diet, a subset of bags (25.0 kg each) from each batch of the basal diet were added to the mixer along with treatment-specific ingredients to achieve the final dietary treatments (Table 3). Dietary treatments were fed in meal form for 21 d.

Pens of pigs were weighed and feed disappearance was recorded on d 0 and 21 to determine average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**).

Serum Myo-inositol and Bone Ash

On d 21 of the study, pigs with body weights closest to the average pen weights were selected and blood samples were collected with subsequent separation of serum. The serum samples were frozen at -20°C and sent on dry ice for analysis (Analytical Services, DSM Nutritional Products Inc., Kaiseraugst, Switzerland) for serum myo-inositol analysis. Serum myo-inositol samples were analyzed by ultra-performance liquid chromatography/mass spectrometry according to the method of Leung et al. (2011).

The same pigs were euthanized via penetrating captive bolt. The right fibula and femur were removed to determine percentage bone ash criteria. Bones were individually placed in a zip-lock plastic bag with a permanent identification tag within the bag and stored at -20°C until analysis. On the day of processing, bones were autoclaved for one hour at 121°C. Femurs and fibulas were cleaned of extraneous soft tissue and placed in a 105°C drying oven for 7 d to determine the dry weight. Bones were then ashed in a muffle furnace at 600°C for 24 h to determine the percentage ash. Ash is expressed as a percentage of dried bone weight.

Chemical and Phytase Activity Analysis

Representative diet samples were obtained from all feeders of each treatment in the first and third weeks of the trial. These samples were combined within dietary treatment to create a composite sample. Four subsamples of the composite samples from each diet, weighing approximately 200 g, were immediately sent for phytase analysis (Analytical Services, DSM Nutritional Products Inc., Belvidere, NJ) as described previously. Subsamples were also analyzed to determine phytase activity at the New Jersey Feed Laboratory Inc. (Trenton, NJ) using AOAC (2009) method (300.24). Final reported values represent the average of the results from both laboratories. The remainder of the composite samples were stored at -20°C at the Kansas State University Swine Laboratory, Manhattan, KS until analysis (Cumberland Valley Analytical Services, Waynesboro, PA). Samples were analyzed for dry matter (DM; method 930.15; AOAC International, 2000), crude protein (CP; method 990.03; AOAC International, 2000), ash (method 942.05, AOAC International, 2000), and ether extract (method 2003.05, AOAC International, 2006). Calcium and P were analyzed using AOAC (2000) method (985.01), with modifications for ashing a 0.35 g sample for 1 h at 535°C, digestion in an open crucible for 20 min in 15% nitric acid on a hot plate, and sample dilution to 50 mL and analysis on an

inductively coupled plasma spectrometer (PerkinElmer 3300 XL and 5300 DV ICP; PerkinElmer Inc., Shelton, CT).

Statistical Analysis

Data were analyzed using the GLIMMIX procedure of SAS (Version 9.3, SAS Institute Inc., Cary, NC). Residual phytase activity was analyzed as a 3 x 2 factorial structure following a binomial distribution with repeated measurements over time. First-order autoregression was selected based on the Bayesian Information Criterion and used to model the covariance structure. This covariance structure implies equal time intervals and a decrease in the correlation as the observations become more separated in time. Interactive and main effects of phytase source, storage form, and storage time were included as fixed effects in the model. If the interaction was significant, differences were determined by using the preplanned pairwise comparisons with the SLICEDIFF option and the Bonferroni multiplicity adjustment. Linear and quadratic polynomial contrasts were implemented to determine the functional form of increasing storage time on residual phytase activity. The growth study consisted of a randomized complete block design, with pen as the experimental unit and BW as the blocking factor. Least square means were calculated for each response variable. When treatment was a significant source of variation, differences were determined by the preplanned pairwise comparisons (PDIFF option) using the Tukey-Kramer multiplicity adjustment to control for Type I Error. For bone ash response, the main effects of bone type and treatment as well as their interaction were tested. In addition, a non-orthogonal contrast was built to compare storing phytases in pure form or in VTM premix. Results were considered significant at $P \leq 0.05$.

RESULTS

Storage Period

Analyzed phytase activities of HiPhos, Axtra Phy, and Quantum Blue in pure forms were 115, 77, and 94% of their stated phytase activity according to the manufacturer's declaration on d 0 prior to storage, respectively (Table 4). When the phytases were mixed in a VTM premix, the analyzed phytase activities were 109, 89, and 103% of their stated phytase activity according to the manufacturer's declaration on d 0 prior to storage for HiPhos, Axtra Phy, and Quantum Blue, respectively.

No interactive effects between phytase source, storage form, and storage time were significant ($P > 0.05$; Table 5), except for the main effects of storage time and storage form. Stability of phytases decreased (linear, $P < 0.05$) as duration of storage increased under high temperature and high humidity until d 90 (Figure 1). Stability of phytases was greater ($P < 0.05$) when they were stored in pure form compared to a VTM form (Figure 1).

Chemical and Phytase Activity Analysis of Experimental Diets

Average values of analyzed P and Ca were higher than formulated values but followed similar patterns as the designed treatment structure (Table 3). The analyzed DM, CP, ether extract, and ash were consistent with formulated values.

As expected, the NC and PC treatments had phytase below the detection limit of the assay (Table 6). The analyzed phytase activity of feed samples in the first week of the growth trial were 76, 94, and 51% of their calculated activity to release 0.15% aP based on the manufacturer declared phytase activity for HiPhos, Axtra Phy, and Quantum Blue stored in pure forms, respectively. The third week samples of HiPhos, Axtra Phy, and Quantum Blue stored in pure form had 80, 72, and 53% of the manufacturer minimum declared phytase activity,

respectively. When phytase sources were stored for 90 d in a VTM premix, the analyzed phytase activity in the first week of the growth trial were 89, 84, and 60% of their calculated activity to release 0.15% aP based on the manufacturer declared phytase activity for HiPhos, Axtra Phy, and Quantum Blue, respectively. Third week samples of HiPhos, Axtra Phy, and Quantum Blue stored in VTM premix form had 73, 71, and 55% of the manufacturer minimum declared phytase activity, respectively.

Growth Trial Period

Pigs fed the PC diet had greater ($P < 0.05$) ADG, ADFI, G:F, d 21 BW, and d 21 bone ash compared to those fed the NC diet (Table 7). Overall, pigs fed the PC diet had greater ($P < 0.05$) ADG compared to pigs fed Axtra Phy stored for 90 d in a VTM premix with no evidence of difference between pigs fed PC and pigs fed the other treatments. There was no evidence of difference in ADFI among pigs fed the PC, or any of the diets containing phytase. For G:F, there was no evidence for difference between the PC and the phytase sources with the exception of Quantum Blue stored in a VTM for 90 d, which has lower ($P < 0.05$) G:F compared to the PC. There was no evidence for difference in final BW between pigs fed the PC and pigs fed the diets containing phytase. There was no evidence ($P > 0.05$) of a bone type by treatment interaction. Bone ash was greater ($P > 0.05$) in femur samples compared to fibula samples (43.5 vs 42.5%, respectively). There was no evidence for difference in bone mineralization for pigs fed the PC diet compared to those fed the HiPhos stored in pure form, while bone mineralization was greater ($P < 0.05$) for those fed the PC compared to the other treatments containing phytase. Furthermore, bone mineralization was lower ($P < 0.05$) for pigs fed the Quantum Blue stored in a VTM for 90 d compared to those fed HiPhos either in pure or VTM form. No evidence for differences was observed for serum myo-inositol concentration.

A specific preplanned, non-orthogonal contrast was completed to compare storage of phytases in pure form versus storage in VTM premixes (Table 7). No evidence of differences ($P > 0.05$) was observed when comparing the average of the three phytase sources stored for 90 d in pure form to the average of the three phytase sources stored for 90 d in VTM for any response criteria.

DISCUSSION

Excess heat, moisture, and mechanical pressure during feed processing can negatively influence the stability of phytases (Jongbloed and Kemme, 1990, Wyss et al., 1998, Ward, 2002). Thus, these factors have the potential to cause a significant reduction in the phytase activity upon denaturation of the enzyme. Briefly, phytase has a proteolytic structure (Yao et al., 2011) that can be unfolded to a disordered polypeptide, with loss of functionality or structure stabilization if under a denaturing influence (Iyer and Ananthanarayan, 2008). Due to its widespread use in the feed industry, attention has been focused on improving the thermostability of this enzyme to bear the high temperatures and moisture during feed processing (Slominski et al., 2007). However, not only the feed processing but also storage form, storage temperature, and storage duration are among factors that can influence the potential for phytase degradation (Sulabo et al., 2011, De Jong et al., 2016). The current study aimed to provide more information regarding the stability of phytase as influenced by storage conditions.

The initial phytase activities on d 0 of storage differed from the calculated values. The analyzed phytase activities were 115, 77, and 94% of the calculated values based on the manufacturer's declared phytase concentration for HiPhos, Axtra Phy, and Quantum Blue stored in pure form, respectively. Similarly, when stored in a VTM premix, the analyzed activities of HiPhos, Axtra Phy, and Quantum Blue were 109,89, and 103% of calculated values,

respectively. These discrepancies between analyzed and calculated phytase activities have also been reported in studies by Sulabo et al. (2011) and De Jong et al. (2016, 2017). The reason for a greater analyzed concentration observed for HiPhos and Quantum Blue in VTM form may be due to an overage of phytase compared with the level declared by the manufacturer to account for potential losses during feed processing and storage (Sulabo et al., 2011). The phytase activity declared by the product manufacturer and used to determine the calculated phytase activities is based on internal assays by each company (De Jong et al., 2016). Differences between the internal assays by each company could also have contributed to variation in the analyzed concentrations when comparing the analyzed values for d 0 to the calculated values. It has also been hypothesized that the coating, which is the case of HiPhos and Axtra Phy, may interfere with the laboratory quantitative analysis (De Jong et al., 2016).

In our study, we observed that phytase activity decreased as storage time increased. Moreover, the current study attempted to simulate storage conditions during summer months with sustained high temperature and humidity. The decrease in phytase activity observed herein is in agreement with results from Sulabo et al. (2011), who observed lower retention rates for phytases stored at 37°C regardless of product and storage form. Similarly, DeJong et al. (2016) reported greater stability of different commercially available phytases for products stored between 4 and 22°C compared to greater temperatures during storage. The detrimental effects of high temperatures on phytase activity have also been reported for other feed additive enzymes (El-Sherniby and El-Chaghaby, 2011).

Shelf-life studies for different phytase sources are reported by the manufacturers in product registrations (European Food Safety Authority, 2012, 2013, 2016). According to these reports, after a 6 to 18-month storage at 25°C, HiPhos recovery was greater than 90% in pure

form and greater than 80% in VTM mixture. The residual phytase activities after 11 to 18 months of storage at 25°C and 60% humidity were greater than 77.5, and 70% for Axtra Phy, and Quantum Blue, respectively. These recovery rates were greater than what was observed in the current study during a 3-month storage. It is worth noting that not only the temperature but also the humidity was higher in our study compared to those used in the registration documents. When Axtra Phy and Quantum Blue were stored at 40°C and 75% humidity, the phytase recovery after 18 months was reported at 28.6% for Axtra Phy, while 21 to 50% recovery was reported for Quantum Blue after a month (European Food Safety Authority, 2013, 2016). These results are more similar to the observed recovered phytase activities of 42.7 to 60.0% among the phytase products in the study herein. Moreover, humidity has been suggested to influence the stability of phytase (Sulabo et al., 2011). According to Iyer and Ananthanarayan (2008), the higher the humidity, the lower the temperature needed to denature an enzyme. Yang et al. (2007) studied the effects of increasing humidity from 53 to 90% under 40°C for 70 d of storage and also observed significant reductions in phytase stability as ambient humidity increased.

When stored at 29.4°C with 75% humidity for 90 d, Quantum Blue stored in pure form had the greatest residual phytase activity, while Axtra Phy stored in VTM premix had the lowest residual phytase activity. This is in accordance with results from De Jong et al. (2016), which indicated that Axtra Phy TPT was less stable at high temperatures and long storage times compared to HiPhos GT, Microtech 5000 Plus, and Quantum Blue G. Coating has been suggested to improve phytase stability during storage (Sulabo et al., 2011). However, in the current study, Quantum Blue stored in pure form had greater stability while being the only uncoated phytase source. The possibility that coating may interfere with the assay methodology has been raised (De Jong et al., 2016), but further research is needed to confirm this influence.

In studies conducted by Sulabo et al. (2011) and Naves et al. (2012), the authors observed that phytase was less stable when stored in a VTM premix. Similar observations were reported by De Jong et al. (2016) who observed that the presence of vitamins and minerals resulted in a larger denaturation effect on phytase compared to product stored either in vitamin premix or in pure form. It is hypothesized that phytase is more likely to interact with inorganic trace minerals in the VTM premix (Shurson et al., 2011). In addition, Lu et al. (2013) appointed the copper (**Cu**) source as the main cause of reduced phytase activity when stored in VTM. The authors observed that phytase retention was greater when Cu was added in the form of tri-basic copper chloride compared to copper sulfate (**$CuSO_4$**), which corroborates with results from Liu et a. (2005) in poultry feed. Results from the current study are in accordance with the aforementioned findings, with phytase sources stored in pure form having greater phytase stability during storage compared to phytases stored in VTM. The VTM premix utilized in the current study contained Cu in the form of $CuSO_4$, which could have resulted in the decreased stability observed for the phytases mixed into the VTM.

The fundamental benefit of a phytase product consists on its ability to improve the P digestibility and therefore increase its availability to the pig. Nutritionists commonly utilize the declared phytase activity from the product label in formulation rather than the phytase activity at the time the product is used for ease in diet formulation. It was previously demonstrated that storage conditions can significantly affect phytase stability. This study also aimed to determine whether the analyzed phytase activity values after 90 d of storage under high temperature and humidity indeed correlates to changes in pig performance. The declared concentration by each manufacturer was used in formulation, with the inclusion of each phytase product determined by the manufacturer to release 0.15% aP. The analyzed phytase activities in the feed samples from

the first and third weeks of the growth trial, however, were greater than expected considering the phytase activity after 90 d of storage. Analysis of phytase in complete diets is typically more variable and less reproducible than analysis in concentrated products (Kim and Lei, 2005). Therefore, we assume the difference is due to analytical variation or difficulties analyzing the phytase in a feed matrix.

Feeding P deficient diets to pigs have been previously shown to cause detrimental effects on growth performance and bone mineralization (Nicodemo et al., 1998, Ruan et al., 2007, Adeola et al., 2015). To the best of our knowledge, there is no research evaluating the effects of different phytase storage conditions on performance of nursery pigs. Exogenous phytase supplementation has consistently been demonstrated to improve P digestibility resulting in increased amount of P to the pig and increased performance (Harper et al., 1997, Kornegay, 2001, Wu et al., 2018). In the current study, growth rate was improved for pigs fed diets containing phytase compared to pigs fed the NC diet deficient in P. Moreover, growth rate did not differ from pigs fed the PC diets with the exception of Axtra Phy stored in VTM. Similarly, feed efficiency was improved for pigs fed the PC and HiPhos stored in pure form compared to pigs fed the NC diets, with pigs fed the other phytase treatments intermediate. These results support the findings that Axtra Phy stored in VTM was the least stable phytase under the storage conditions used in this study.

According to Crenshaw et al. (2009), the femur provides a better fit to dietary P concentration for assessment of whole-body mineral content of growing pigs compared to the fibula. In our study, we observed that, in fact, the bone mineralization was greater in the femur compared to the fibula. However, these differences due to bone type did not influence the dietary treatment responses. With the exception of HiPhos stored in pure form, storing phytases for 90 d

under high humidity and temperature resulted in decreased bone mineralization compared to the PC. This was to be expected because of the lowered phytase activity after the 90-d storage period. The practical implications are that storage conditions should be accounted for in the diet formulation to obtain equivalent bone mineralization properties as the PC control diet.

Plasma myo-inositol has previously been shown to increase with the addition of phytase in the diet (Guggenbuhl et al., 2016). It is speculated that plasma myo-inositol is involved in pig growth rate and bone osteogenesis (Croze and Soulage, 2013, Cowieson et al., 2015). Even though numerical improvements in serum myo-inositol concentration compared to NC were observed for the PC and phytase supplemented diets, no evidence for differences among treatments were detected in our study.

Understanding the effects of storage conditions on phytase stability is important to maximize pig performance and avoid P deficiencies. In conclusion, this study demonstrated that phytase activity decreases as duration of storage in high temperature and high humidity conditions increases. Moreover, retained phytase activity was decreased when they were stored as part of a VTM premix compared to phytase sources stored in pure form. Bone ash was also reduced when phytases were stored for 90 d compared to the PC, with the exception of HiPhos stored in pure form.

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Table 5.1 Composition of the phytase-free vitamin and trace mineral (VTM) premix used in the study[1]

Item	Amount/kg
Vitamin	
Vitamin A, IU	6,666,667
Vitamin D3, IU	1,333,334
Vitamin E, IU	66,667
Vitamin K, mg	2,667
Riboflavin, mg	6,667
Niacin, mg	30,000
Pantothenic acid, mg	22,000
Cobalamin, mg	30
Folic acid, mg	2,000
Thiamine, mg	2,000
Pyridoxine, mg	2,667
Biotin	200
Trace mineral	
Copper (CuSO4), mg	4,536
Iodine [Ca(IO3)2], mg	303
Iron (FeSO4), mg	45,359
Manganese (MnO2), mg	16,633
Selenium (Selenium yeast), mg	91
Zinc (ZnSO4), mg	33,248

[1]The amount added for each phytase product was determined such that including 0.15% premix in the diet would provide the phytase recommended by their respective manufacturers to release 0.15% available phosphorus [(1000 FYT/kg feed HiPhos, DSM Nutritional Products, Parsippany, NJ); (651 FTU/kg feed Axtra Phy, Dupont, Wilmington, DE); and (500 FTU/kg feed Quantum Blue, AB Vista, Plantation, FL)].

Table 5.2 Diet composition of basal diet (as-fed basis)[1]

Item	Basal diet
Ingredient, %	
Corn[2]	61.22
Soybean meal, 46,5% crude protein[2]	36.37
Calcium carbonate	1.04
Monocalcium phosphate, 21%[2]	0.19
Sodium chloride	0.65
L-Lysine-HCl	0.29
DL-Methionine	0.14
L-Threonine	0.10
Total	100.00
Calculated analysis	
SID[3] amino acids	
Lysine	1.30
Isoleucine:lysine	64
Leucine:lysine	128
Methionine:lysine	34
Methionine & cysteine:lysine	58
Threonine:lysine	62
Tryptophan:lysine	19.1
Valine:lysine	69
Total lysine, %	1.46
Net energy, kcal/kg	2,414
Crude protein, %	22.8
Calcium, %	0.54
Phosphorus, %	0.47
Available phosphorus, %	0.12

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

[2] A total of 3 samples of corn, boybean meal, and monocalcium phosphate were analyzed for P concentration in duplicate (Ward Laboratories, Inc., Kearney, NE). The average of the six lab results for each ingredient was used for diet formulation, which corresponded to 0.31, 0.66, and 20.54% for corn, soybean meal, and monocalcium phosphate, respectively.

[3] Standardized ileal digestible.

Table 5.3 Diet composition of experimental diets (as-fed basis)[1]

Item	Negative Control	Positive Control	Stored in Pure form[1]			Stored in VTM premix form[2]		
			HiPhos	Axtra Phy	Quantum Blue	HiPhos	Axtra Phy	Quantum Blue
Ingredient, %								
Basal diet	98.95	98.97	98.95	98.95	98.95	98.95	98.95	98.95
Calcium carbonate	-	0.15	-	-	-	-	-	-
Monocalcium phosphate	-	0.73	-	-	-	-	-	-
Sand[3]	0.90	-	0.90	0.90	0.90	0.90	0.90	0.90
VTM premix[4]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis								
Calcium, %	0.54	0.72	0.54	0.54	0.54	0.54	0.54	0.54
Phosphorus, %	0.47	0.62	0.47	0.47	0.47	0.47	0.47	0.47
Available phosphorus, %	0.12	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Chemical analysis[5]								
Calcium, %	0.58	0.83	0.64	0.62	0.60	0.59	0.62	0.62
Phosphorus, %	0.59	0.76	0.55	0.58	0.57	0.57	0.59	0.59

[1] The three sources of phytase (HiPhos GT - coated, Axtra Phy TPT- coated, and Quantum Blue G – uncoated but marketed as heat stable) were stored in a pure form or mixed in a phytase-free VTM and then stored for 90 days in an environmental chamber (29.4°C and 75% humidity) before diet manufacturing.

[2] The three sources of phytase (HiPhos GT - coated, Axtra Phy TPT- coated, and Quantum Blue G – uncoated but marketed as heat stable) were mixed in a phytase-free VTM premix and stored for 90 days in an environmental chamber (29.4° C and 75% humidity) before diet manufacturing.

[3] Sand was used to equalize inclusion rates of experimental ingredients.

[4] The negative and positive control diets were formulated with a phytase-free VTM premix. For the other treatments, the amount added for each phytase product was determined such that including 0.15% VTM premix in the diet would provide the activity of phytase recommended by the manufacturer to release 0.15% available P (1000 FYT/kg feed HiPhos, 651 FTU/kg feed Axtra Phy, and 500 FTU/kg feed Quantum Blue).

[5]A representative sample of each diet was collected from 6 feeders, homogenized, and submitted to Cumberland Valley Analytical Services, Waynesboro, PA for chemical analysis. Analysis of dry matter, crude protein, crude fiber, ether extract, and ash were within analytical variation.

Table 5.4 Calculated and analyzed phytase composition of samples at d 0 of storage[1]

	Declared PU[3]/g	AOAC analysis, PU/g	Percentage of minimum declared PU[4]
Pure Product			
HiPhos[5]	20,000	22,940	115
Axtra Phy[6]	20,000	15,524	77
Quantum Blue[7]	40,000	37,592	94
VTM premix			
HiPhos[5]	666	728	109
Axtra Phy[6]	434	388	89
Quantum Blue[7]	333	344	103

[1] Values represent averages of 6 replicates. The AOAC analysis were performed at the DSM Nutritional Products Laboratory (Belvidere, NJ).

[2] The VTM premix without phytase was sampled and analyzed for phytase activity on d 90 and found to be free of phytase.

[3] PU= phytase units. Declared PU according to the manufacturer of each phytase source.

[4] Percentage analyzed phytase activity according to the AOAC analysis relative to the declared phytase activity by the manufacturer.

[5] DSM Nutritional Products, Parsippany, NJ.

[6] Dupont, Wilmington, DE.

[7] AB Vista, Plantation, FL.

Table 5.5 Probabilities of interactive and main effects of phytase source, storage form, and storage time on phytase stability (as defined by percentage of initial phytase activity) of commercially available phytase products

Item	*P*-value
Interactive effect	
Storage time × storage form × phytase source	0.130
Storage time × phytase source	0.092
Storage time × storage form	0.727
Storage form × phytase source	0.121
Main effect	
Storage time	<0.001
Storage form	0.019
Phytase source	0.363

Table 5.6 Calculated and analyzed phytase composition of feed samples at first and third week of the growth trial period[1]

Item	Phytase Composition				
	Calculated PU[2]/kg feed	AOAC analysis, PU/kg feed		Percentage of calculated PU/kg feed[3]	
		First week	Third Week	First Week	Third Week
Negative Control[4]	0	<50	<50	-	-
Positive Control[5]	0	<50	<50	-	-
Pure product[6]					
HiPhos[7]	1,000	759	769	76	80
Axtra Phy[8]	651	613	474	94	72
Quantum Blue[9]	500	257	267	51	53
VTM premix[10]					
HiPhos[7]	1,000	890	727	89	73
AXTRA PHY[8]	651	548	459	84	71
Quantum Blue[9]	500	300	275	60	55

[1] Dietary samples were collected in the first and third week of the growth trial, and values represent averages of 8 replicates. The AOAC analysis were performed at the DSM Nutritional Products Laboratory (Belvidere, NJ) and at the New Jersey Feed LaboratoryInc., (Trenton, NJ).

[2] PU= phytase units. Calculated values represent the amount of PU of each phytase source needed to release 0.15% aP based on the manufacturer declared phytase activity on d 0 prior to storage.

[3] Percentage analyzed phytase activity according to the AOAC analysis relative to the calculated PU/kg of feed based on the declared phytase activity by the manufacturer.

[4] The negative control diet was formulated to 0.12% aP provided by monocalcium phosphate.

[5] The positive control diet was formulated to 0.27% aP provided by monocalcium phosphate.

[6] The three sources of phytase (HiPhos, Axtra Phy, and Quantum Blue) were added to the diets to release 0.15% aP. They were stored in a pure form for 90 days in an environmental chamber (29.4°C and 75% humidity) before diet manufacturing.

[7] DSM Nutritional Products, Parsippany, NJ.

[8] Dupont, Wilmington, DE.

[9] AB Vista, Plantation, FL.

[10] The three sources of phytase (HiPhos, Axtra Phy, and Quantum Blue) were added to the diets to release 0.15% aP. They were mixed in a phytase-free VTM premix and stored for 90 days in an environmental chamber (29.4°C and 75% humidity) before diet manufacturing.

Table 5.7 Effects of phytase when stored in a concentrated VTM premix or as a pure product on growth performance and bone mineralization of nursery pigs[1]

			Stored in Pure form[2]			Stored in VTM form[2]				Probability, *P* =	
Item[3]	Negative Control[4]	Positive Control[4]	HiPhos	Axtra Phy	Quantum Blue	HiPhos	Axtra Phy	Quantum Blue	SEM	Overall[5]	Stored in VTM vs Pure[6]
d 0 to 21											
ADG, g	484^{c}	644^{a}	640^{a,b}	625^{a,b}	585^{a,b}	611^{a,b}	575^{b}	605^{a,b}	23.4	<0.001	0.106
ADFI, g	868^{b}	991^{a}	983^{a}	1,012^{a}	975^{a}	967^{a}	962^{a,b}	1,018^{a}	41.3	<0.001	0.660
G:F	558^{a}	649^{c}	651^{c}	617^{b,c}	603^{a,b,c}	634^{b,c}	600^{a,b,c}	597^{a,b}	11.4	<0.001	0.155
Body weight, kg											
d 0	11.7	11.7	11.8	11.7	11.7	11.8	11.7	11.8	0.66	0.999	0.987
d 21	22.2^{b}	25.3^{a}	25.2^{a}	24.8^{a}	24.0^{a}	24.6^{a}	24.2^{a}	24.4^{a}	1.05	<0.001	0.360
Bone ash, %											
Femur + fibula[7]	38.4^{d}	46.9^{a}	44.6^{a,b}	43.3^{b,c}	42.8^{b,c}	44.1^{b}	42.8^{b,c}	41.3^{c}	0.64	<0.001	0.125
Serum myo-inositol											
µg/ml	14.6	17.5	16.2	17.9	15.3	18.1	19.0	16.4	1.13	0.074	0.113

[1] A total of 300 pigs (DNA, 241 × 600, initial pen average body weight 11.7 kg) were used in a 21-d growth study with 4 or 5 pigs per pen, and 8 pens per treatment. All pigs were fed a diet deficient in phosphorus (0.12% aP) for 4 days prior to the initiation of the trial. On the last day of the trial, 1 pig per pen (average weight of the pen) was selected and a blood sample was collected, with subsequent separation of serum, which was sent for myo-inositol analysis (DSM Nutritional Products, Kaiseraugst, Switzerland). These same pigs were humanely euthanized via captive bolt. The right fibula and femur were removed from euthanized pigs to determine percentage bone ash criteria.

[2] The three sources of phytase (Hiphos, Axtra Phy, And Quantum Blue) were added to the diet in order to release 0.15% aP for a 0.15% premix inclusion in the diet. They were stored for 90 days in a pure form or in a VTM premix form in an environmental chamber (29.4°C and 75% humidity) before diet manufacturing.

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

[4] The negative control diet was formulated to 0.12% aP provided by monocalcium phosphate. The positive control diet was formulated to 0.27% aP provided by monocalcium phosphate.

[5] All possible pairwise comparisons were protected by the Tukey-Krummer adjustment. Different superscripts within a column differ ($P < 0.05$).

[6] This contrast compared the average of the three phytase sources stored for 90 d in pure form to the average of the three phytase sources stored for 90 d in VTM premix.

[7] There was no significant ($P > 0.05$) interaction between dietary treatment and bone type. Bone ash was greater ($P < 0.05$) for femur compared to fibula samples (43.5 vs 42.5%, respectively).

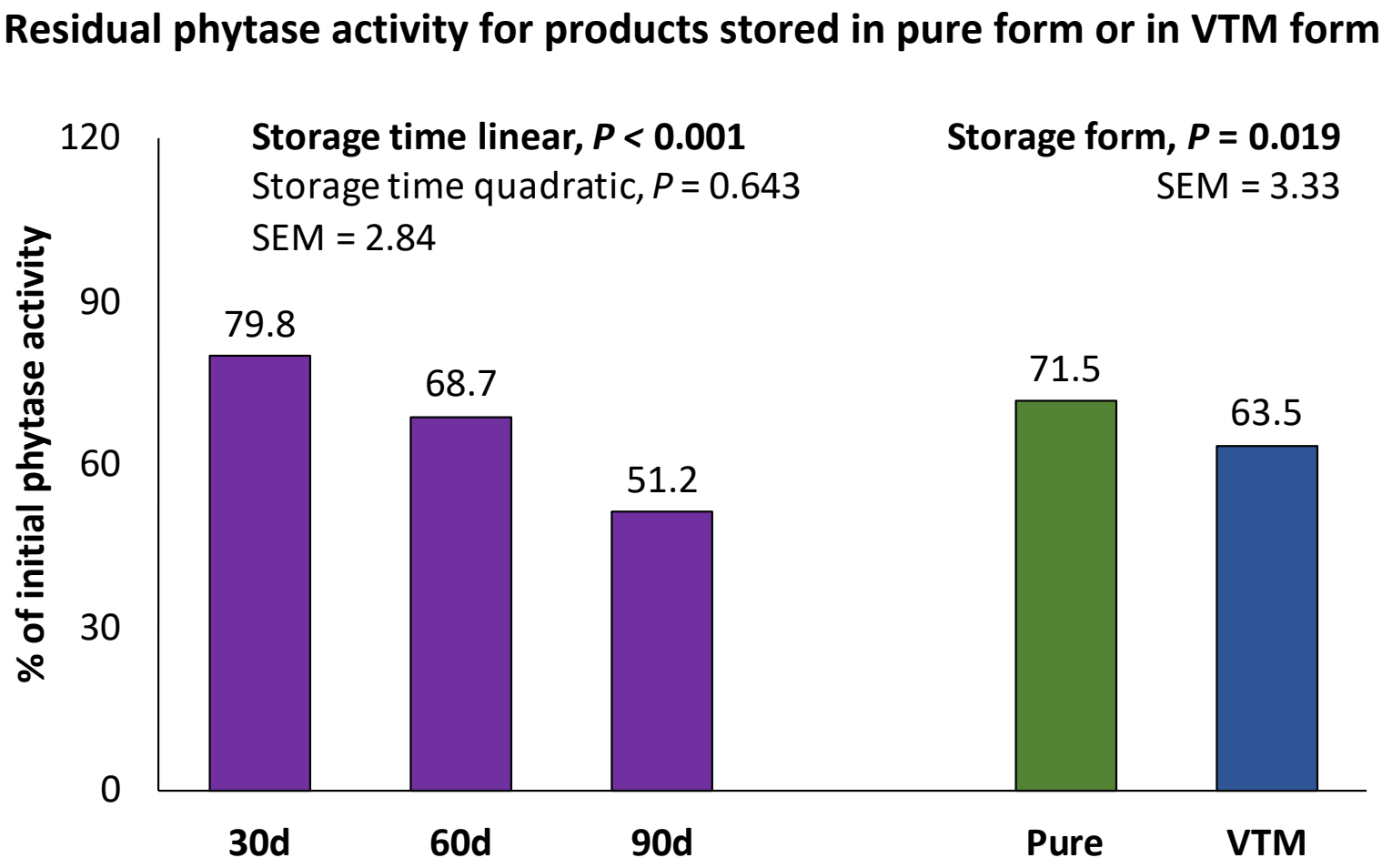

Figure 5.1 Residual phytase activity (% of initial activity on d 0 prior to storage) for HiPhos GT (DSM Nutritional Products, Parsippany, NJ), Axtra Phy TPT (Dupont, Wilmington, DE), and Quantum Blue G (AB Vista, Plantation, FL) as affected by storage time (30 to 90 d) and (pure and vitamin and trace mineral premix **(VTM)**) when stored in a controlled environmental chamber set at

29.4°C and 75% humidity. Stability of phytases decreased ($P < 0.05$) as duration of storage increased. Stability of phytases were greater ($P < 0.05$) when they were stored in pure form compared to VTM form.

Chapter 6 - A survival guide to reproducible research for the animal sciences

ABSTRACT

Reproducibility of research results refers to the ability to reproduce a study's findings given the original data and code used to analyze the data, and it is the foundation of scientific progress. There is wide agreement among the scientific community that reproducibility of research findings is lower than desirable. In addition, research findings lacking reproducibility are unlikely to be replicated through an independent experiment and generalized to a larger population. This article focuses on reproducibility in the animal sciences from the aspects of making the raw data available, documenting the statistical model, and reporting that is integrated with the statistical analysis. Researchers are unlikely to share data and code in the absence of formal requirements. Several reasons may contribute to this reluctance of making scientific findings transparent. In this article, we emphasized the importance and the benefits of a culture that values open science. Moreover, we provide different tools for storing data and code in a data repository to make them publicly available. Access to research materials is as important as an accurate description of the data and material and methods to improve reproducibility and transparency of research results. Many times, the description of the statistical methodology in a manuscript is not specific enough for an independent researcher to recreate the study findings. The method of literate programming is an attempt to increase comprehensibility and to make programming language human readable by tying explanations to instructions. It integrates statistical and reporting packages, enabling the incorporation of plain text sentences in conjunction with computational language. An appendix file is available to illustrate how to write a fully reproducible report in a manuscript format utilizing tools from an open source statistical

software. Specifically, a comprehensive documentation and step-by-step reporting of the methodology implemented in data organization and analysis is described. Overall, several reproducible research tools are available to make data and code publicly accessible in a data repository, and to generate dynamic reports that accurately describe the steps involved in generating the research findings. The adoption of these research practices constitutes achievable steps towards improving transparency standards, credibility of scientific claims, and, ultimately, research reproducibility for the animal sciences.

Keywords: data repository, reproducible research tools, statistical reporting

INTRODUCTION

The foundation of scientific progress relies on the reproducibility of research results. The credibility of scientific claims is enhanced by the replication of these claims (Open Science Collaboration, 2015). In view of the variation and inconsistency in reproducible research terminology, this article follows the definitions of Patil et al. (2016). Reproducibility refers to the ability to reproduce a study's findings given the original data and code used to analyze the data. Replicability refers to the ability to obtain consistent estimates from an independent repetition of a study with the same methodology but without the original data. Therefore, we will focus on reproducibility from the aspects of making the raw data available, documenting the statistical model, and reporting that is integrated with the statistical analysis.

A survey on reproducibility including more than 1,500 scientists has identified that over 70% of the researchers failed to reproduce other scientists' results (Baker, 2016). Moreover, more than 50% of the researchers themselves were unable to reproduce the results of their own experiments. The amount of data quantifying the extent and scope of reproducibility issues in the scientific literature is scarce. However, greater attention has been recently given to the

irreproducibility of published research findings (Prinz et al., 2011, Begley and Ellis, 2012, Peers et al., 2012). Begley and Iodannis (2015) compiled studies from high-profile journals and empirically estimated that the reproducibility rate of research results in the preclinical field ranged from 10 to 25%, which is similar to the rate of 15% estimated for research in the biomedical field.

The extent to which these findings apply to other scientific disciplines is unknown. Nevertheless, this inability to reproduce research results is not exclusive to the preclinical and biomedical fields, but rather it has a much broader spectrum (Nuzzo, 2015, Baker, 2016). In fact, irreproducibility concerns have been recently raised and discussed in the animal sciences (Bello, 2016, Bello and Renter, 2018). These discussions, however, regarded the correct use of statistical concepts and proper experimental design. The objectives of the current article are to 1) illustrate reasons leading to irreproducibility of scientific results related to data and code management and reporting, and 2) provide general recommendations and describe tools to aid in the dissemination of reproducible research.

OPEN SCIENCE

There is a wide agreement among the scientific community that reproducibility of research findings is lower than desirable (Begley and Iodannis, 2015, Munafò et al., 2017, Bello and Renter, 2018). Research findings lacking reproducibility, that is, the ability to obtain the same results given the same data set and codes, are unlikely to be replicated through an independent experiment and generalized to a larger population. (Schloss, 2018). Moreover, reproducibility does not guarantee correctness but is the one thing that can be effectively assured in a published study (Broman et al., 2017). A study evaluated the reproducibility of data analyses from microarray-based gene studies published in Nature Genetics in 2005-2006 (Iodannis et al.,

2009). The authors were able to reproduce data of only 2 of 18 analyzed papers. The attributed reason for the low reproducibility of results observed in this study was the unavailability of the original raw data used to generate the results of the studies evaluated. Furthermore, reanalysis of data from 127 microarray-based gene studies published between 2011 and 2012 revealed a frequent lack of support to the original studies' claims (Witwer 2018).

Open science refers to the process of allowing the content and process that originated the evidence and claims from research studies to be accessible to others (Mufanò et al., 2017). Accumulated evidence reinforces the importance of access to original raw data and statistical codes. The lack of availability of the materials used to generate research results limits the ability of others to reproduce and build upon them. Yet, science often lacks transparency and openness. A study evaluating articles published in 2014 from high impact journals in the disciplines of biology, chemistry, mathematics, and physics observed that only 13% of the articles make the data available to others (Womack, 2015). Similar issues have been discussed regarding the availability of statistical codes (Morin et al., 2012).

Researchers are unlikely to share data and code in the absence of formal requirements. Several reasons may contribute to the reluctance of making scientific findings transparent. Challenges to expand the adoption of data and code sharing are technical and social. Several disciplines lack a culture that values open science and code and data are not recognized as research materials (Broman et al., 2017). Other reasons may include a lack of enforcement, paucity of data and code sharing related incentives, fear of scrutiny or misinterpretation, and competition (Wichert et al., 2006, Savage and Vickers, 2009, Longo and Drazen, 2016). Technical aspects of research like end-to-end scripting is time consuming. In addition, there may

be lack of awareness of the tools available to document reproducible research (Broman et al., 2017).

Summaries from meta-analyses, systematic reviews, and randomized controlled trials are considered the strongest evidence base for the medical and veterinary practices (Sauerland and Seiler, 2005). We believe the animal science disciplines have in general wide-spread adoption of randomized controlled trials and sound experimental methods. Examples include the implementation of randomized designed experiments with blocking factors and implementation of mixed models (Littell et al., 1998, Gonçalves et al., 2016). We also believe that the animal science community has history of utilizing systematic reviews. Excellent examples of these are the National Research Council (NRC) reports on nutrient requirements across the various species. These are widely viewed as the baseline for standard of practice in the nutrition communities. Additionally, we have published several meta-analysis summaries (Nitikanchanna et al., 2015, Paulk et al., 2015, Flohr et al., 2018). A challenge in conducting meta-analysis is that, in most cases, we have had to limit the observational unit to the treatment means reported. We have enhanced this process by weighting observations (St-Pierre, 2001) proportional to the inverse of the standard error. This indirectly weights the observations by the sample size since the standard error is influenced by sample size. However, the underlying data is rarely available for analysis.

The peer-review process of subjecting a researcher's study to the scrutiny of peer experts in the same field is at the core of high-quality science. This article is an extension of a peer-review and provides tools to improve this process. The benefits of openness and transparency among the scientific communities go beyond the facilitation of research reproducibility and validation. The habit of publicly sharing data and code likely results in higher quality code and

better levels of documentation that allows the authors themselves as well as others to reproduce the analyses and build upon them (Stodden et al., 2016). Furthermore, scientists can ask new questions from existing data, and data can be combined in methods that increase its value, as an example in the development of meta-analysis studies (Borgman, 2012). Data and code sharing can be assigned a digital object identifier, which may also increase the recognition of authors and the number of citations in addition to the paper itself (Gewin, 2016).

The culture of making research data and software code publicly available may also elicit unexpected and welcome collaborations and feedbacks that can enhance statistical methods. Because statistical approaches evolve over time, current best statistical practices may not be adequate in the future. As an example, basic or classical statistical approaches are not well suited to fit models with nonnormal data (e.g., proportions or counts) and in the presence of random effects; instead, generalized linear mixed models are the best tool to analyze nonnormal data that involve random effects (Bolker et al., 2008). Thus, making research data available to others enables future reanalysis to be performed applying the newest and more appropriate statistical methods that are developed.

The benefits described above have stimulated many journals and organizations to adopt policies to support and promote public access to research data. The Public Library of Science (PLOS) is among the first large and influential journals to implement open data and open code requirements (PLOS, 2018). Nature and Science, the two highest ranked journals in scientific publication, adopted similar policies that require the research data underlying the claims and conclusions of the reported research findings to be available, preferably via public repositories. These guidelines were expanded to include not only the original raw data but code and algorithms upon request (AAAS, 2018, Nature, 2018). The National Institutes of Health (NIH)

also requires research dataset disclosure and encourages code availability, and the National Science Foundation requests submission of a data management plan that includes how data will be stored and shared.

Journals and publishers have an influential role towards improving reproducibility, transparency standards, and scientific reliability (Lin and Strasser, 2014). However, even when data or code sharing are required by journal policy or society ethical standards, requests for data accessibility are often unfulfilled (Vanpaemel et al., 2015, Wicherts et al., 2016). Moreover, accessibility of original raw data and other research materials decreases over time (Vines et al., 2014). According to Nosek (2015), there are pragmatic barriers and challenges to sharing and few incentives to overcome them. In fact, promising examples include the adoption of incentives such as badges to acknowledge open practices (Kidwell et al., 2016). Badges would be given to authors who meet certain open science criteria, such as code and data sharing. They correspond to a symbol at the top of a paper to explicitly recognize that the authors value transparency. According to Kidwell et al. (2016), the proportion of publications with open data increased by 38% after the implementation of badges.

Research data are frequently difficult to obtain if they are not stored and publicly available in a data repository (Federer et al., 2018). There are different tools for data and code sharing available. Specialized and field specific repositories include, for example, the Protein Data Bank for protein structures, and GenBank for gene sequences data. Public Library of Science identified and provided a list of digital repositories for field specific data or for cross-disciplinary data. The latter includes data repositories like Open Science Framework (OSF), Harvard Dataverse Network, figshare, Dryad Digital Repository, and Zenodo. Simple yet powerful platforms for publishing and sharing code are also available (Kubilius, 2014). The code

sharing service from GitHub enables automatic revision control, assigning a revision number for each code modification. In addition to GitHub, the figshare service has the ability to assign a digital object identifier to the code, which may help to enhance recognition for open code contributions. Other tools similar in functionality to the popular GitHub for code publication are OSF, Zenodo, Banyan, and Scigit. These online repositories examples constitute practical and achievable methods to embrace transparency, reproducibility and the reusability of research products. We have provided an example raw data set and statistical code utilized to analyze the data, which are available on OSF (Vier, 2019). The raw data a statistical code can be accessed at: https://osf.io/mcqfa/?view_only=46ba9b74a4f1409a80770c75222e5168.

TOOLS FOR REPRODUCIBLE RESEARCH

An accurate description of the material and methods and the data is important to improve the reproducibility and transparency of research results (Gorgolewski and Poldrack, 2016). However, most times the description of the statistical methodology in a manuscript is not specific enough for an independent researcher to recreate the research results. Programming errors, lack of adequate documentation regarding the executions involved in the analysis and transfer of results values from statistical packages into manuscripts can also lead to irreproducibility. In addition, the use of spreadsheets and other data organization and analysis tools do not provide a friendly and reusable audit system to trace and describe how results were obtained. Therefore, to successfully share data and code, a detailed report file tracking and explaining all of the steps involved in the data preparation and data analysis should be generated (Russo et al., 2016).

According to Mesirov (2010), a reproducible research system involves two components. The first component consists of a reproducible research environment with the statistical tools

required to perform data analysis and the ability to document each step for redistribution. The second component consists of a reproducible research publisher with tools to construct dynamic reports in a narrative fashion and that is easily linked to the reproducible research environment.

The typical workflow starts with a statistical software package (e.g. SAS, Stata, R, Python) to perform the data analysis and a layout package (e.g. Microsoft Word, Microsoft PowerPoint, portable document format) to translate the results into a written report or presentation (Baumer et al., 2014). However, this type of workflow is not completely integrated, and more prone to potentially introducing errors and hence, irreproducibility. Knuth (1984) introduced the concept of literate programming as an attempt to increase comprehensibility and to make programming language human readable by tying explanations to instructions.

The method of literate programming integrates statistical and reporting packages, enabling the incorporation of plain text sentences in conjunction with computational language. *Code chunks* consist of sequences of commands in a specific programming language (Gentleman and Lang, 2007). They are responsible to execute the computations required to generate the outputs reported in a publication. *Text chunks* consist of a narrative sequence of plain text sentences to describe the methodology, the code, the outputs produced and sometimes their interpretation (Gentleman and Lang, 2007). They are formatted in a way that expresses and describes computational details in a textual manner to benefit both authors and readers. Thus, lines of codes are augmented with comments and explanatory sentences, enhancing transparency and reliability of the results obtained, as well as facilitating knowledge transfer (Russo et al., 2016).

There are several statistical software tools available supporting literate programming, connecting reproducible research environment and reproducible research publisher features. R (R

Foundation for Statistical Computing, Vienna, Austria) is an open source software that contains several easy-to-use tools that enable literate programming and facilitate writing these dynamic documents. Traditionally, literate programming tools are founded on the macro package LaTeX documentation system as an authoring environment. The LaTeX system produces high-quality typesetting in a plain text form and creates finished reports by compiling the text files into different output formats. The *Sweave* system is a method to integrate executed R code into LaTeX documents (Leisch, 2002). Substantial improvements were made to ease the creation of dynamic documents in R for those who are not familiar with the LaTeX language (Gandrud, 2016). R Markdown and the *knitr* package provide similar functionality to *Sweave;* however, with a simpler syntax to produce high quality reproducible reports (Xie, 2015, Allaire et al., 2016). These reports can be rendered through a third-party software such as Pandoc in different formats such as hypertext markup language (HTML), portable document format (PDF), and docx compatible with Microsoft Word.

SASweave provides literate programming capability for SAS (SAS Institute Inc., Cary, NC) in a similar way that *Sweave* does for R (Lenth and Højsgaard, 2007). Thus, SAS code is embedded into a LaTeX document containing outputs and documentation together. This document can be post-processed and rendered into a PDF document. Another option for SAS users to create reproducible results documents is using the StatRep package, which is also based on the LaTeX typesetting system (Arnold and Kuhfeld, 2012). The system reads both the code and markup and generates an executable SAS program file. This program file includes SAS macros and the output delivery system document to capture the output as external files, which can then be compiled into a PDF document.

MarkDoc, *Ketchup*, and *Weaver* packages are features that support literate programming in Stata (Haghish, 2014; Stata Corp, College Station, Texas). These packages support the LaTeX and HTML typesetting systems, as well as a simpler markup language called Markdown. They consist of user-written packages to enable the creation of dynamic documents that combine Stata codes, output, and text. These dynamic documents can be further exported into a variety of file formats, including PDF and docx compatible with Microsoft Word. Several tools are available for Python (Python Software Foundation) users to create reproducible documents with embedded code. Among others, *PythonTeX* and *Pweave*, a Python version of *Sweave*, allow code and outputs to be embedded with either LaTeX or Markdown documents, and to be parsed into different document formats (Poore, 2015).

Jupyter, an interactive web application, is another literate programming tool that was originally designed for the Python programming language (Pérez and Granger, 2007). It is becoming more popular because many different programming languages such as Julia and R are now supported to create notebooks that combine executable code, rendered visualizations, and descriptive text in a single interactive and portable document (Shen, 2014). Thus, it is possible to combine strengths of different programming languages and save the interactive documents in various output formats, including HTML and PDF. Researchers work with different statistical software tools, but the principles of literate programming can be applicable to analyses involving several computational languages as illustrated above. Regardless of the statistical software utilized to run the data analysis, it is important to include information about the specific version of the program used and other critical tools (Ellis and Leek, 2018).

We have deposited a data set in an open source data repository (Vier, 2019), developed R code to analyze the data, documented the statistical model and then provided the report using R

Markdown and the *knitr* package. Thus, providing an integrated open source reproducible document, which is also publicly available (Vier, 2019). Therefore, the raw data is available for others to analyze in a different format, and the source code is available to provide the tables, graphs, and explanations. Also, the reporting format is such that the output can be reorganized to change the units from imperial to metric system or vice versa. Thus, practitioners and other researchers will be able to manipulate the output into customizable formats for different audiences. A commented R code is available as an appendix file (see Appendix A) to illustrate how to write a fully reproducible report in a manuscript format utilizing R tools.

Besides explicitly linking computational code, results, and narrative into an organized and dynamic document, the report should be publicly available. Upon accepting the material for journal publication, some specific form of license is likely required (Gentleman and Lang, 2007). The terms of the license file indicate the use of this document and its contents by the journal, the author, and the readers. Data are not protected by copyright law in many jurisdictions (Marwick et al., 2018). The Creative Commons Public Domain declaration (CC-0) is recommended by Stodden (2009) for data, while the Creative Commons Attribution (CC-BY) may be more suitable for other documentation such as articles and figures. Open source software licenses are designed for software and may not always be appropriate for data and dynamic reports (Marwick et al., 2018).

In addition to licensing, another related issue is version control. The use of hosted version control in statistical practice facilitates collaboration among colleagues with powerful tools for managing versions, as well as the distribution and maintenance of the material (Ram, 2013, Bryan, 2018). Many researchers use an informal version control with derivative copy creation, differentiating the file version with initials, dates, or other descriptors (Bryan, 2018). This

method ultimately leads to multiple file versions. Git is a version control system example that manages the evolution and records changes to a set of files in a structured manner, preserving the computational code change history in a report. Researchers can track the data analysis and reporting development and revert to old versions. Git is similar to the track changes feature in a word processing program, but more powerful. GitHub is a widely used cloud storage service functioning as a host and acting as a distributor for a Git-managed project, while also offering control over who can see and edit a project (Gandrud, 2013, Bryan, 2018).

CONCLUSION

The reproducibility of research findings is the core of scientific progress. Yet, science often lacks transparency and openness. The need for open science, including access to raw data and the statistical codes is essential for reproducible research practices in the animal sciences. A lack of availability of the materials used to generate the claimed research results affects their credibility and limits the ability of others to reproduce and build upon them. There is also a need for comprehensive documentation practices and step-by-step reporting of the methodology implemented in data organization and analysis.

Efforts to address these systematic issues and to expand the adoption of open data and open code will require tremendous commitment from researchers, journals, institutions, and funding agencies. Several reproducible research tools are available to make data and code publicly accessible in a data repository, and to generate dynamic reports that accurately describe the steps involved in generating the research findings. The adoption of these research practices constitutes achievable steps towards improving transparency standards, credibility of scientific claims, and, ultimately, research reproducibility.

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Appendix A - Supplement R code from Chapter 6

USE OF R FOR REPRODUCIBLE REPORTS

R is an open source software that has an active development community and is constantly expanding the applications capabilities. It contains several easy-to-use tools that enable literate programming and facilitate writing these dynamic documents. R Markdown and the *knitr* package have a simple markup to produce high quality reproducible reports. These reports can be rendered in different formats through a third-party software Pandoc, such as hypertext markup language (**HTML**), portable document format (**PDF**), and docx compatible with Microsoft Word. We illustrate how to write a fully reproducible analysis in a manuscript format utilizing R tools.

R Markdown

R Markdown is a simple-to-use system to create enriched R files (typically saved with the Rmd extension) written in Markdown markup language for making dynamic documents. These files are designed to be used with the *rmarkdown* package and constitute well-annotated source files to generate reproducible documents, including an *ain't markdown language* header (**YAML**), codes, comments, tables, and figures. The lines of code are reported in a self-contained way, known as *code chunks*, which are preceded by explanatory comments to describe the ideas underlying their implementation (Russo et al., 2016). The R Markdown files can be transformed through the *knitr* package (Xie 2015). The *knitr* package identifies, runs, and compiles all the *code chunks* through R, incorporating their outputs in a report saved in an md extension. Subsequently, *rmarkdown* relies on a utility program called *pandoc* that renders the reports in different formats, such as HTML, PDF, and docx compatible with Microsoft Word.

The R codes are highlighted and followed by their outputs in the transformed report, facilitating their understanding.

Header and explanatory sentences

An easier way to create an R-Markdown script is by using the R Studio, an R development environment tool integrated with literate programming tools. A header, written in YAML, can be included in the generated script specifying information regarding the document's title, author, and how to render the document. Three dashes (---) are used to begin and end a header, and a colon (:) separates the information keys from their associated descriptions. The title, author, and date are located in the beginning of the report document.

```
---
title: "Example of YAML header"
author: Carine Vier
date: 10/17/18
output: pdf_document
---
```

Markdown language is utilized to write the explanatory comments throughout the report document. This markup is a simple and straightforward set of conventions designed for formatting plain text (e.g. *italicized* text is surrounded by an asterisk, and **bold** text is surrounded by two asterisks), and the majority of its syntax is available in a short web page (R Studio, 2015).

Code chunks

Lines of statistical programming language to be included in the markup report documents are typically contained in a code chunk. The R markdown syntax to begin and end a code chunk is (```{r}) and (```), respectively, and arguments of R coded are included in between. To

explicitly assign chunk code labels in R markdown documents, the label is placed inside the braces after the letter *r* (e.g. ```{r label_name}). There are several options to customize the presentation of the code chunks in the rendered report (Table 1). Customization includes options to display both code and results from the code in the rendered report, or to only show the output of a code command such as a table or figure. The code chunk options are specified in the chunk head and placed after the chunk label, separated by a comma (e.g. ```{r label_name, echo=TRUE} to display the code in the reported document). To insert a comment within a code chunk, a hashtag identifies it as a plain text instead of a line of code.

```
```{r label_name, echo=TRUE}
# Insert lines of code in this space
```
```

If a code chunk option is to be applied to all the chunks in the document, a global code chunk is set. Global chunk options aid in the consistency of formatting throughout the document without the need of specifying similar options repetitively. The *opts_chunk$set* command is included as an argument within a code chunk in the beginning of the document (e.g. opts_chunk$set(fig.allign=`center`) to centralize all the figures in the rendered document).

```
```{r Global Chunk, echo=FALSE}
opts_chunk$set(fig.align='center')
```
```

Inline code and results

Inline allows for R code or output to be displayed with the plain text in the report document. Therefore, results can be dynamically reported within the text, automatically changing when the data or the model changes. A static inline code can be included in the Markdown document with the code enclosed in single backticks (e.g. `static code is inserted here`). A

dynamic inline code can be included in the Markdown text with the code enclosed by single backticks followed by the letter *r* (e.g. `r dynamic code is inserted here`).

Results in tables

To avoid the potential error introduction through manually typing results into tables, tables in the rendered report can be dynamically connected to the statistical results. Different tools are available in R for creating dynamic tables with the Markdown markup, such as the *xtable* package, the *texreg* package, and the *pandoc* package. All these packages have capabilities to produce tables from data frames and objects that contain the results from statistical models fitted to the data. Setting the option *results='asis'* enables the tables created to be compiled as markup tables and included in the rendered report.

Different statistical models applied to a data set in R can be saved in a model object. The linear model command (*lm*), for example, fits a simple linear regression model and creates model summaries of the *lm* class. The coefficient estimates, standard error, and *p*-values from the *lm* model object can be summarized in a table using the *xtable* package, as follows:

```
```{r lm_table, results='asis', echo=TRUE}

# Fit a linear regression model and save it to a model object
lm_object <- lm(ADG ~ days_to_market, data=pig_growth)

# Create an xtable object from the lm object
lm_table <- xtable(lm_object, caption="Linear Regression Model fo
r ADG",
                   label="BasicXtableSummary")

# Print out the output of the xtable object created
print.xtable(lm_table, caption.placement=”top”)

```
```

The *texreg* package to create tables supports a greater number of model object types when compared to the *xtable* package. Another advantage of the *texreg* package is that it allows

output tables to be created with the inclusion of estimates from multiple statistical models. Different functions, *screenreg*, *texreg*, and *htmlreg* can be used to create the tables. The *screenreg* typesets the table in plain-text format; *texreg* typesets the table in LaTeX format; and the *htmlreg* typesets the table in HTML format, allowing the table to be opened in Microsoft Word.

```
```{r lm_table, results='asis', echo=TRUE}

# Fit two linear regression models and save them to model objects
lm_object1 <- lm(ADG ~ days_to_market, data=pig_growth)
lm_object2 <- lm(ADG ~ days_to_market + pneumonia_score, data=pig
_growth)

# Create customized coefficient names from the model objects crea
ted
cust_coef <- c('(Intercept)', 'Days to Market', 'Pneumonia Score'
)

# Create a table from the model objects created to an HTML format
htmlreg(list(lm_object1, lm_object2), caption ="Nested Linear Reg
ression Models for ADG", doctype=TRUE,
 caption.above=TRUE, custom.coef.names=cust_coef)

```
```

The *pander* package provides a simple and easy tool for transforming and rendering tabular R objects into markdown tables. It is somewhat similar to the *texreg* package; however, its greater advantage is that it works closely with *pandoc*. Thus, the tabular R objects do not have to be manually transformed to markdown objects, and the resulted rendered tables are automatically transformed to different formats like PDF, HTML, and Word documents.

```
```{r MixedModel_table, results='asis', echo=TRUE}

#Fitting a mixed model to the ADG response variable.
adgmodel <- lmer(ADG_grams~treatment + (1|block), REML=T, data=reproduc
ible)

#Fitting the ANOVA to the mixed model.
adganova <- Anova(adgmodel)
```
```

```
#Obtaining lsmeans.
adg.lsm <- emmeans(adgmodel, "treatment")

#Pairwise comparisons.
adg.pwc<- cld(adg.lsm, reversed=T, Letters=letters, adjust="Tukey", alp
ha=.05)

adg.pwc2<-data.frame(Treatment=adg.pwc$treatment, Mean=adg.pwc$emmean,
SE=adg.pwc$SE,Letter=adg.pwc$.group)



#Creating a table with the lsmeans, SEM, and grouped letters that can b
e #rendered to any format (HTML,PDF, Word)
pander(adg.pwc2, keep.line.breaks = TRUE,caption = "Mean ADG as affecte
d by treatment, (alpha=0.05).")
```

Results in graphs

R contains a comprehensive set of data visualization tools. These tools enable the incorporation of dynamic graphs to visually display information in the rendered documents. The *graphics* package is the default package for creating graphs in R. This package includes several commands to create several types of plots (e.g. *hist* for histograms, *boxplot* for boxplots, and *plot* for scatterplots). Murrell's (2011) book is a useful resource to learn how to implement the R default's graphics capabilities. A package developed more recently, *ggplot2*, is the most popular system for creating graphics. It expands the capabilities and aesthetic customizations of the default's R *graphics* package. There are good resources available for learning how to utilize the *ggplot2* package (Wickham, 2009, Chang, 2012).

Plots generated with the *ggplot2* package include different layers, such as the variables plotted and labels. Aesthetic elements for each layer are defined by the *aes* argument. The main layer type is known as geometric, and it defines the type of plot created by the *geom* argument, including lines, points, and bars, for example. The process to include graphs in knitted reports is similar to the process for including tables.

```{r scatterplot, results='asis' echo=TRUE}

ggplot(data=pig_growth, aes(x=days_to_market, y=ADG) +
geom_point(shape=16, size=3, show.legend=FALSE) +
theme_minimal()+
scale_color_gradient()

```

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Table A.6.1 *knitr* code chunk options[1]

Code chunk option label	Type	Description
cache	logical	Whether or not to save results from the code chunk in a cache database.
eval	logical	Whether or not to run the code.
echo	logical	Whether or not to include the code in the rendered document.
error	logical	Whether or not to include error messages.
fig.align	character	Align figures.
include	logical	Whether or not to include the results in the rendered document.
message	logical	Whether or not to include R messages.
out.height	numeric	Set figures' heights in the rendered document.
out.width	numeric	Set figures' widths in the rendered document.
results	character	How to include results in the rendered document.
warning	logical	Whether or not to include R warnings.

[1]Adapted from *Gandrud* (2016).