# Effects of dietary calcium to phosphorus ratio and addition of phytase on growth performance of nursery pigs<sup>1</sup>

# Fangzhou Wu,\*<sup>,2</sup> Mike D. Tokach,\* Steve S. Dritz,<sup>†</sup> Jason C. Woodworth,\* Joel M. DeRouchey,\* Robert D. Goodband,\* Marcio A. D. Gonçalves,<sup>‡</sup> and Jon R. Bergstrom<sup>§</sup>

\*Department of Animal Sciences and Industry, College of Agriculture, Kansas State University, Manhattan 66506; †Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan 66506; ‡Genus PIC, Hendersonville, TN 37075; <sup>§</sup>DSM Nutritional Products Inc., Parsippany, NJ 07054

ABSTRACT: Two studies were conducted to evaluate the growth performance and percentage bone ash of nursery pigs fed various combinations of Ca and P provided by inorganic sources or phytase. In Exp. 1, pens of pigs (n = 720, initially 6.1  $\pm$  0.98 kg) were blocked by initial BW. Within blocks, pens were randomly assigned to one of six treatments (12 pens per treatment) in a three-phase diet regimen. Treatments were arranged in a  $2 \times 3$  factorial with main effects of Ca (0.58% vs. 1.03%) and standardized total tract digestible (STTD) P (0.33% and 0.45% without phytase, and 0.45% with 0.12% of the P released by phytase). During treatment period, Ca × P interactions were observed for all growth criteria (P < 0.05). When diets had low Ca, pigs fed 0.45% STTD P with phytase had greater (P < 0.01) ADG and ADFI than those fed 0.33% or 0.45% STTD P without phytase. When high Ca was fed, ADG and ADFI were similar among pigs fed 0.45% STTD P with or without phytase and were greater than those fed 0.33% STTD P. Gain:feed was reduced (P < 0.01) when high Ca and low STTD P were fed relative to other treatments. On d 21, radiuses were collected from 1 pig per pen for bone ash analysis. Pigs fed 0.33% STTD P had decreased (P < 0.05) percentage bone ash than those fed 0.45% STTD P with or without phytase when high Ca was fed, but

this P effect was not observed for low Ca diets (Ca  $\times$  P interaction, P = 0.007). In Exp. 2, 36 pens (10 pigs per pen, initially  $6.0 \pm 1.08$  kg) were used in a completely randomized design. Treatments were arranged in a  $2 \times 3$  factorial with the main effects of STTD P (at or above NRC [NRC. 2012. Nutrient Requirements of Swine. 11th rev. ed. Washington (DC): National Academic Press.] requirement estimates) and total Ca (0.65, 0.90, and 1.20%). Experimental diets were fed during phases 1 and 2, followed by a common phase 3 diet. Diets at NRC (2012) P level contained 0.45% and 0.40% STTD P, compared with 0.56% and 0.52% for diets greater than the NRC (2012) estimates, in phase 1 and 2, respectively. During treatment period, increasing Ca decreased (linear, P = 0.006) ADG, but increasing STTD P marginally increased (P = 0.084)ADG, with no  $Ca \times P$  interaction. When diets contained NRC (2012) P levels, pigs fed 1.20% Ca had decreased (P < 0.05) G:F than those fed 0.65% or 0.90% Ca; however, when high STTD P were fed, G:F was not affected by Ca (Ca  $\times$  P interaction, P = 0.018). In conclusion, excess Ca decreased pig growth and percentage bone ash when diets were at or below NRC (2012) requirement for STTD P, but these negative effects were alleviated by adding monocalcium P or phytase to the diet.

Key words: bone ash, calcium, growth performance, nursery pig, phosphorus, phytase

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#### INTRODUCTION

Appropriate dietary Ca and P concentrations are essential for nursery pig performance. Accurate formulation for Ca and P is even more important in recent years with the routine use of phytase in swine diets. Research has demonstrated that feeding excess dietary Ca impairs P absorption, resulting in reduced growth performance and bone calcification of pigs (Reinhart and Mahan, 1986; Stein et al., 2011; González-Vega et al., 2016). This effect is especially prominent when diets are marginal in P (Létourneau-Montminy et al., 2012; NRC, 2012). Moreover, excess Ca can bind to phytate in the small intestine, decrease the solubility of phytate, and therefore impair the ability of phytase to release P (Dersjant-Li et al., 2015).

Diets can have excess Ca for multiple reasons, including formulation errors, variability in laboratory analysis of ingredients, neglecting the Ca content of carriers in premixes or other additives, and not accounting for Ca released by phytase. Meanwhile, diets can also be deficient in P due to formulation errors or by overestimating the amount of P released for the given amount of phytase in the diet. In addition, recent research (Vier et al., 2017) has suggested that NRC (2012) may underestimate the standardized total tract digestible (STTD) P requirement for nursery pigs. Thus, in commercial production there is an increased risk of overfeeding Ca. The effects of dietary Ca and P concentration as well as their ratio on growth performance and P retention have been extensively studied in growing-finishing pigs. However, to our knowledge, such information is limited for pigs less than 15 kg BW. Therefore, the objective of this study was to evaluate the growth performance and percentage bone ash of early nursery pigs in response to different combinations of dietary STTD P and Ca concentrations provided by monocalcium P or phytase.

# MATERIALS AND METHODS

All experimental procedures in this study were approved by the Kansas State University Institutional Animal Care and Use Committee (Manhattan, KS).

#### Animals and Housing

Two studies were conducted at the Cooperative Research Farm's Swine Research Nursery (Kalmbach Feeds, Inc., Sycamore, OH). Each pen  $(1.52 \times 1.83 \text{ m}^2)$  had slated metal floors and was equipped with a four-hole stainless-steel feeder and a nipple-cup waterer. Five barrows and five gilts (PIC 280 × Camborough, Genus PIC, Hendersonville, TN) were housed in each pen and were allowed ad libitum access to feed and water throughout the experiments. In Exp. 1, 720 weaned pigs were used from two rooms with 36 pens per room. Upon arrival, pigs were individually weighed and assigned to pens in order to achieve balanced pen weights within room. After 4 d of adaptation, pens of pigs were blocked by BW (initial pig BW =  $6.1 \pm 0.98$  kg) and allotted randomly to one of six dietary treatments (Tables 2 and 3). In Exp. 2, 360 weaned pigs with initial BW of  $6.0 \pm 1.08$  kg were housed in a single room with 36 pens. Pens of pigs were allotted to one of six dietary treatments (Tables 4 and 5) in a completely randomized manner.

#### Diets and Experimental Design

All ingredients containing Ca and P were sampled and sent to two labs (Ward Laboratories, Inc. Kearney, NE; and Cumberland Valley Analytical Services Inc., Maugansville, MD) for analysis of Ca and P in duplicate in each lab (Table 1). The average of the four lab results for each ingredient was used in diet formulation in both experiments. In Exp. 1, the six dietary treatments were arranged in a 2  $\times$  3 factorial, with two levels of Ca (0.58%) and 1.03%) and three levels of STTD P (0.33% with no phytase, 0.45 with no phytase, and 0.45% with 0.12% of the P assumed to be released by phytase). Diets with phytase contained 1,000 phytase units (FYT) of Ronozyme HiPhos 2500 (DSM Nutritional Products, Inc., Parsippany, NJ) with an assumed releasing value of 0.12% for Ca and STTD P. Pigs were fed in three phases, with the experimental diets provided in phases 1 (d 0 to 14) and 2 (d 14 to 28). A common phase 3 diet was then fed to all pigs from d 28 to 42. Nutrient and standardized ileal digestible AA digestibility coefficients used for diet formulation were obtained from NRC (2012). All diets were provided in meal form.

In Exp. 2, the six dietary treatments were arranged in a  $2 \times 3$  factorial with two levels of STTD P (at or above NRC (2012) requirement estimates) and three levels of total Ca (0.65, 0.90, and 1.20%). Pigs were fed in three phases with the experimental diets provided in phases 1 (d 0 to 10) and 2 (d 10 to 24), followed by a common phase 3 diet from d 24 to 45. Diets formulated to meet NRC (2012) P requirement (NRC) contained 0.45% and 0.40% STTD P in phases 1 and 2, respectively. Diets formulated to exceed NRC (2012) P requirement

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

|                          |        | Ca, %              |         | P, %  |       |         |  |
|--------------------------|--------|--------------------|---------|-------|-------|---------|--|
|                          | Lab 1* | Lab 2 <sup>†</sup> | Average | Lab 1 | Lab 2 | Average |  |
| Corn                     | 0.06   | 0.02               | 0.04    | 0.28  | 0.23  | 0.26    |  |
| Soybean meal             | 0.35   | 0.31               | 0.33    | 0.63  | 0.62  | 0.62    |  |
| HP 300 <sup>‡</sup>      | 0.39   | 0.37               | 0.38    | 0.72  | 0.72  | 0.72    |  |
| Spray-dried<br>whey      | 0.84   | 0.86               | 0.85    | 0.86  | 0.87  | 0.86    |  |
| Monocalcium<br>P (21% P) | 15.80  | 15.85              | 15.83   | 22.00 | 19.01 | 20.50   |  |
| Limestone                | 36.48  | 39.55              | 38.02   | 0.01  | 0.01  | 0.01    |  |
| Trace mineral premix     | 7.44   | 8.03               | 7.74    | 0.01  | ND§   | 0.01    |  |
| Vitamin premix           | 12.58  | 13.69              | 13.13   | 0.07  | 0.04  | 0.05    |  |
| Phytase <sup>#</sup>     | 11.77  | 12.87              | 12.32   | 0.11  | 0.05  | 0.08    |  |
| Selenium premix          | 35.66  | 40.41              | 38.04   | 0.02  | 0.02  | 0.02    |  |

\*Lab 1 (Ward Laboratories, Inc.); samples were analyzed in duplicates and average values were reported.

<sup>†</sup>Lab 2 (Cumberland Valley Analytical Services Inc.); samples were analyzed in duplicate and average values were reported.

<sup>‡</sup>Hamlet Protein, Inc., Findlay, OH.

§Not detectable.

\*Ronozyme HiPhos 2500 (DSM Nutritional Products, Inc., Parsippany, NJ).

(>NRC) contained 0.56% and 0.52% STTD P in phases 1 and 2, respectively. Diets did not contain phytase with the dietary Ca and P mainly provided by monocalcium phosphate and limestone. Phase 1 diets were prepared in pellet form and phases 2 and 3 diets were provided in meal form. Pigs and feeders were weighed at the end of each feeding phase to determine ADG, ADFI, and G:F ratio in both of the experiments.

## **Bone** Ash Analysis

On d 21 of Exp. 1, one median-weight gilt from each pen was euthanized using a CO<sub>2</sub> chamber and radiuses were collected. Bones were then transferred on dry ice to the Kansas State University Swine Laboratory and stored at  $-20^{\circ}$ C until analysis. After thawing at room temperature (24°C) in plastic bags for 24 h, bones were autoclaved for 60 min, adhering tissue and cartilage caps were removed, then dried at 105°C for 7 d. Dried radiuses were ashed in a muffle furnace at 600°C for 24 h to determine total ash weight and percentage bone ash.

### **Chemical Analysis**

Complete diet samples were obtained and delivered to the Kansas State University Swine Laboratory, Manhattan, KS, and stored at -20°C

until analysis. Feed samples were analyzed for DM, CP, ether extract, Ca, and P at Ward Laboratories, Inc. Concentrations of Ca and P in complete feed samples were also analyzed at Cumberland Valley Analytical Services Inc. and Midwest Laboratories (Omaha, NE) in duplicate. Standard procedures from AOAC (2006) were followed for analysis of moisture (Method 934.01), CP (Method 990.03), ether extract (Method 920.39), Ca and P (Method 985.01). At Cumberland Valley Analytical Services Inc., AOAC (2000) method (985.01) was used for Ca and P analyses with modifications of 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

Experiment 1 was analyzed in a randomized completely block design with a  $2 \times 3$  factorial treatment structure. The statistical model contained the main effects of Ca and STTD P and their interactions as well as random effects of room and weight block within room. The initial statistical model included treatment and the effect of treatment within room as fixed effects. Because there was no evidence that the treatment effect was different across rooms, the treatment within room term was removed from the model and data from the two rooms were pooled in the analyses of growth performance and percentage bone ash. One pen from 0.58% Ca + 0.45% STTD P treatment encountered issues with feeder adjustment and had restricted feed intake as noted in the daily observation records; therefore, data from this pen were excluded from all the analyses. In Exp. 2, data were analyzed in a completely randomized design with a  $2 \times 3$  factorial treatment structure. The statistical model contained the main effects of STTD P and Ca and their interaction. Single degree-of-freedom contrasts were performed to test the linear and quadratic effects of increasing Ca and their interactions with P concentration. All statistical analyses were conducted using the GLIMMIX procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. Means were reported as least-squares means. For response criteria with significant Ca  $\times$ P interaction, means were separated by the PDIFF option with a Tukey-Kramer adjustment. Results were considered significant at P < 0.05 and marginally significant at 0.05 < P < 0.10.

#### RESULTS

#### **Chemical Analysis**

Analyzed Ca concentrations in feed ingredients were similar between the two laboratories (Table 1). However, a 15% interlaboratory discrepancy was observed for monocalcium phosphate, the primary source of P in the experimental diets; therefore, the average values were used in the diet formulation. It is worthy to note that significant amounts of Ca were included in minor ingredients, such as vitamin and trace mineral premixes, phytase, and selenium premix. However, given the small inclusion rates, these ingredients only contributed 0.03% total Ca in the experimental diets. The analyzed dietary Ca and P concentrations were slightly greater than the formulated values but followed similar patterns as the designed treatment structure (Tables 2–5).

## **Experiment** 1

During phase 1 (d 0 to 14; Table 6),  $Ca \times P$  interactions were observed for ADG and G:F (P < 0.05) but not for ADFI. Pigs fed diets containing 0.45% STTD P with phytase had greater (P < 0.01) ADG than pigs fed 0.45% STTD P without phytase or pigs fed 0.33% STTD P regardless of dietary Ca concentration. The ADG of pigs fed diets containing 0.45% STTD P without phytase was greater (P < 0.001) than that of pigs fed 0.33% STTD P when diet contained high (1.03%) Ca concentration but not for diets with low (0.58%) Ca concentration. Regardless of Ca level, feeding 0.45% STTD P with phytase improved (P < 0.05) ADFI compared with diets with 0.33% or 0.45% STTD P with no phytase. Pigs fed 0.45% STTD P with or without phytase exhibited greater (P < 0.10) G:F than pigs fed 0.33% STTD P, and the magnitude of these differences was more prominent when diets contained high Ca concentrations (Ca × P interaction, P < 0.001).

During phase 2 (d 14 to 28), Ca × P interactions were observed for all growth criteria (P < 0.05). Pigs fed diets containing 0.45% STTD P with or without phytase had greater (P < 0.05) ADG than those fed 0.33% STTD P when high Ca was added to diets but not for diets containing low Ca concentrations. When diets contained low Ca, feeding 0.45% STTD P with phytase resulted in greater (P < 0.001) ADFI than feeding the 0.33% STTD P diet, with ADFI of pigs fed 0.45% STTD P without phytase intermediate. When fed high Ca, ADFI of pigs fed 0.45% STTD P with or without phytase was greater (P < 0.01) than those fed 0.33% STTD P. Pigs fed 0.33% STTD P had lower (P < 0.001) G:F than those fed 0.45% STTD P without phytase when diets contained high Ca concentration; however, no differences were observed among low Ca diets.

When combining the treatment periods (d 0 to 28), Ca  $\times$  P interactions were observed for all growth responses (P < 0.05). When low Ca was added to diets, feeding 0.45% STTD P with phytase increased (P < 0.01) ADG and ADFI compared with pigs fed 0.45% STTD P without phytase and pigs fed 0.33% STTD P. However, with high Ca, ADG and ADFI were similar among pigs fed 0.45% STTD P with or without phytase but were greater than those fed 0.33% STTD P diet. Gain to feed was decreased (P < 0.01) when low STTD P and high Ca were added to the diet compared with other dietary treatments. On d 28, when diets contained low Ca concentrations, pigs fed 0.45% STTD P with phytase had greater (P < 0.01) BW than pigs fed 0.45% STTD P without phytase and those fed 0.33% STTD P. When diets contained high Ca, BW was similar among pigs fed 0.45% STTD P with or without phytase but was greater (P < 0.01) than those fed 0.33% STTD P diet.

During the posttreatment period from d 28 to 42, all pigs received a common phase 3 diet. No evidence for significant Ca  $\times$  P interaction was observed for ADG. Pigs previously fed 1.03% Ca had greater (P < 0.001) ADG than those previously fed 0.58% Ca. Pigs previously fed 0.33% STTD P tended to have greater (P = 0.054) ADG than those previously fed 0.45% STTD P with phytase, but similar ADG to pigs previously fed 0.45% STTD P without phytase. Pigs previously fed 0.45% STTD P with or without phytase had greater (P < 0.05) ADFI than those previously fed 0.33% STTD P, but the magnitude of these differences was greater in high Ca than in low Ca diets (Ca  $\times$  P interaction, P = 0.063). For G:F, a Ca × P interaction (P < 0.001) was observed. When diets contained low Ca concentration, pigs previously fed 0.45% STTD P with phytase had decreased (P = 0.027) G:F compared with those previously fed 0.33% STTD P, with G:F of pigs previously fed 0.45% STTD P without phytase intermediate. When high Ca was added to diets, G:F was similar among pigs previously fed 0.45% STTD P with or without phytase but was poorer (P < 0.01) than those previously fed 0.33% STTD P.

Overall (d 0 to 42), Ca × P interaction was observed for all growth criteria (P < 0.10). Feeding 0.33% STTD P decreased (P < 0.01) ADG compared with feeding 0.45% STTD P with or without

| Table 2. Diet formulation, | phase 1 | (Exp. 1 | ; as-fed | basis) |
|----------------------------|---------|---------|----------|--------|
|----------------------------|---------|---------|----------|--------|

| Ca, $\%$ :0.580.580.58*1.031.03STTD P, no phytase, $\%$ :0.330.450.330.330.45STTD P, with phytase, $\%$ :0.45Ingredients, $\%$ 0.45Corn44.0243.3244.4941.8641.16Soybean meal25.1825.2325.1525.3325.38HP 300†6.006.006.006.006.00Spray-dried whey20.0020.0020.0020.0020.00Beef tallow2.202.452.053.003.25   | 1.03*<br>0.33<br>0.45<br>42.40<br>25.29<br>6.00<br>20.00<br>2.80<br>0.20<br>1.47<br>0.45<br>0.38 |
|--|--|
| STTD P, no phytase, %: $0.33$ $0.45$ $0.33$ $0.33$ $0.45$ STTD P, with phytase, %: $0.45$ Ingredients, %Corn $44.02$ $43.32$ $44.49$ $41.86$ $41.16$ Soybean meal $25.18$ $25.23$ $25.15$ $25.33$ $25.38$ HP 300 <sup>†</sup> $6.00$ $6.00$ $6.00$ $6.00$ $6.00$ Spray-dried whey $20.00$ $20.00$ $20.00$ $20.00$ $20.00$ Beef tallow $2.20$ $2.45$ $2.05$ $3.00$ $3.25$   | 0.33<br>0.45<br>42.40<br>25.29<br>6.00<br>20.00<br>2.80<br>0.20<br>1.47<br>0.45<br>0.38          |
| STTD P, with phytase, %:         -         -         0.45         -         -           Ingredients, %         -         -         0.45         -         -         -           Corn         44.02         43.32         44.49         41.86         41.16           Soybean meal         25.18         25.23         25.15         25.33         25.38           HP 300 <sup>†</sup> 6.00         6.00         6.00         6.00         6.00           Spray-dried whey         20.00         20.00         20.00         20.00         20.00           Beef tallow         2.20         2.45         2.05         3.00         3.25 | 0.45<br>42.40<br>25.29<br>6.00<br>20.00<br>2.80<br>0.20<br>1.47<br>0.45<br>0.38                  |
| Ingredients, %Corn $44.02$ $43.32$ $44.49$ $41.86$ $41.16$ Soybean meal $25.18$ $25.23$ $25.15$ $25.33$ $25.38$ HP 300 <sup>†</sup> $6.00$ $6.00$ $6.00$ $6.00$ Spray-dried whey $20.00$ $20.00$ $20.00$ $20.00$ Beef tallow $2.20$ $2.45$ $2.05$ $3.00$   | 42.40<br>25.29<br>6.00<br>20.00<br>2.80<br>0.20<br>1.47<br>0.45<br>0.38                          |
| Corn $44.02$ $43.32$ $44.49$ $41.86$ $41.16$ Soybean meal $25.18$ $25.23$ $25.15$ $25.33$ $25.38$ HP $300^{\dagger}$ $6.00$ $6.00$ $6.00$ $6.00$ Spray-dried whey $20.00$ $20.00$ $20.00$ $20.00$ Beef tallow $2.20$ $2.45$ $2.05$ $3.00$  | 42.40<br>25.29<br>6.00<br>20.00<br>2.80<br>0.20<br>1.47<br>0.45<br>0.38                          |
| Soybean meal $25.18$ $25.23$ $25.15$ $25.33$ $25.38$ HP $300^{\dagger}$ $6.00$ $6.00$ $6.00$ $6.00$ Spray-dried whey $20.00$ $20.00$ $20.00$ $20.00$ Beef tallow $2.20$ $2.45$ $2.05$ $3.00$   | 25.29<br>6.00<br>20.00<br>2.80<br>0.20<br>1.47<br>0.45<br>0.38                                   |
| HP 300 <sup>+</sup> 6.00         6.00         6.00         6.00           Spray-dried whey         20.00         20.00         20.00         20.00         20.00           Beef tallow         2.20         2.45         2.05         3.00         3.25  | 6.00<br>20.00<br>2.80<br>0.20<br>1.47<br>0.45<br>0.38  |
| Spray-dried whey20.0020.0020.0020.00Beef tallow2.202.452.053.003.25  | 20.00<br>2.80<br>0.20<br>1.47<br>0.45<br>0.38  |
| Beef tallow         2.20         2.45         2.05         3.00         3.25   | 2.80<br>0.20<br>1.47<br>0.45<br>0.38   |
|  | 0.20<br>1.47<br>0.45<br>0.38   |
| Monocalcium P (21% P) 0.19 0.87 0.19 0.20 0.88   | 1.47<br>0.45<br>0.38   |
| Limestone 0.62 0.34 0.29 1.80 1.52   | 0.45<br>0.38   |
| Salt 0.45 0.45 0.45 0.45   | 0.38   |
| L-Lys HCl 0.38 0.38 0.38 0.38  |  |
| DL-Met 0.23 0.23 0.23 0.23 0.23  | 0.23   |
| L-Thr 0.18 0.18 0.18 0.18 0.18   | 0.18   |
| L-Trp 0.03 0.03 0.03 0.03 0.03   | 0.03   |
| L-Val 0.08 0.08 0.09 0.09  | 0.09   |
| Trace mineral premix <sup>‡</sup> $0.09$ $0.09$ $0.09$ $0.09$ $0.09$   | 0.09   |
| Vitamin premix <sup>§</sup> 0.05 0.05 0.05 0.05  | 0.05   |
| Choline chloride 0.04 0.04 0.04 0.04 0.04  | 0.04   |
| Phytase <sup>#</sup> 0.04  | 0.04   |
| Zinc oxide 0.27 0.27 0.27 0.27 0.27  | 0.27   |
| Selenium 0.02 0.02 0.02 0.02 0.02  | 0.02   |
| Total 100.00 100.00 100.00 100.00 100.00   | 100.00   |
| Calculated composition   |  |
| Standardized ileal digestible AA, %  |  |
| Lys 1.40 1.40 1.40 1.40 1.40   | 1.40   |
| Ile:Lvs 61 61 61 61 61   | 61   |
| Leu:Lvs 116 116 116 115 115  | 115  |
| Met:Lvs 37 37 37 37 37   | 37   |
| Met & Cvs:Lvs 58 58 58 58 58   | 58   |
| Thr:Lvs 65 65 65 65  | 65   |
| Trp:Lys 20 20 20 20 20   | 20   |
| Val:Lvs 70 70 70 70 70 70  | 70   |
| Total Lvs. % 1.53 1.53 1.53 1.53   | 1.53   |
| CP % 21.01 20.98 21.02 20.95 20.92   | 20.96  |
| NE kcal/kg 2 568 2 568 2 569 2 568   | 2.568  |
| Ca no phytase $\%$ 0.58 0.58 0.46 1.03 1.03  | 0.91   |
| Ca with phytase $\%$ 0.58 0.58 0.58 1.03 1.03  | 1.03   |
| STTD P no phytase % 0.33 0.45 0.33 0.45  | 0.33   |
| STTD P with phytase %         0.33         0.45         0.33         0.45  | 0.35   |
| Total P % $0.52$ $0.66$ $0.52$ $0.52$ $0.66$   | 0.52   |
| Analyzed composition %   | 0.52   |
| DM 91 31 92 17 91 76 91 52 91 61   | 91.22  |
| $CP \qquad 21.90 \qquad 20.45 \qquad 22.15 \qquad 21.10 \qquad 21.55$  | 21.22  |
| $ \begin{array}{cccccccccccccccccccccccccccccccccccc$  | 21.40<br>4.65  |
| $\Gamma_{2}$ 0.56 0.65 0.60 0.02 1.00  | 0.87   |
| P <sup>&amp;</sup> 0.61 0.74 0.62 0.67 0.91  |  |

\*Phytase was added to diets at the level of 1,000 phytase units with assumed release value of 0.12% for Ca and STTD P.

<sup>†</sup>Hamlet Protein, Inc., Findlay, OH.

<sup>‡</sup>Provided per kg of premix: 29.6 g Mn from manganese oxide, 104 g Fe from iron sulfate, 112 g Zn from zinc sulfate, 16 g Cu from copper sulfate, 1,600 mg I from calcium iodate.

<sup>8</sup>Provided per kg of premix: 28,659,800 IU vitamin A, 4,409,200 IU vitamin D<sub>3</sub>, 105,821 IU vitamin E, 801,665 mg vitamin K, 15,423 mg riboflavin, 66,138 mg pantothenic acid, 110,230 mg niacin, 79 mg vitamin B<sub>12</sub>, 4,409 mg folic acid, 44 mg thiamin, 44 mg pyridoxine, and 4.4 mg biotin. <sup>#</sup>Ronozyme HiPhos 2500 (DSM Nutritional Products, Inc., Parsippany, NJ).

«Averaged across analyzed values from Ward Laboratories, Inc., Cumberland Valley Analytical Services Inc., and Midwest Laboratories.

Table 3. Diet formulation, phases 2 and 3 (Exp. 1; as-fed basis)\*

| Item                               | Phase 2       |        |                   |        |                            |                   |               |  |  |
|------------------------------------|---------------|--------|-------------------|--------|----------------------------|-------------------|---------------|--|--|
| Ca, %:                             | 0.58          | 0.58   | 0.58 <sup>†</sup> | 1.03   | 1.03                       | 1.03 <sup>†</sup> | 0.86          |  |  |
| STTD P, no phytase, %:             | 0.33          | 0.45   | 0.33              | 0.33   | 0.45                       | 0.33              | 0.37          |  |  |
| STTD P, with phytase, %:           | -             | -      | 0.45              | -      | -                          | 0.45              | 0.47          |  |  |
| Ingredients, %                     |               |        |                   |        |                            |                   |               |  |  |
| Corn                               | 52.18         | 51.48  | 52.70             | 50.04  | 49.34                      | 50.56             | 59.47         |  |  |
| Soybean meal                       | 29.54         | 29.59  | 29.50             | 29.69  | 29.74                      | 29.65             | 35.15         |  |  |
| HP 300 <sup>‡</sup>                | 3.00          | 3.00   | 3.00              | 3.00   | 3.00                       | 3.00              | -             |  |  |
| Spray-dried whey                   | 10.00         | 10.00  | 10.00             | 10.00  | 10.00                      | 10.00             | -             |  |  |
| Beef tallow                        | 2.20          | 2.45   | 2.00              | 3.00   | 3.25                       | 2.80              | 2.00          |  |  |
| Monocalcium P (21% P)              | 0.59          | 1.27   | 0.59              | 0.60   | 1.28                       | 0.60              | 1.22          |  |  |
| Limestone                          | 0.66          | 0.38   | 0.33              | 1.84   | 1.56                       | 1.51              | 1.06          |  |  |
| Salt                               | 0.50          | 0.50   | 0.50              | 0.50   | 0.50                       | 0.50              | 0.35          |  |  |
| L-Lys HCl                          | 0.38          | 0.38   | 0.38              | 0.38   | 0.38                       | 0.38              | 0.29          |  |  |
| DL-Met                             | 0.20          | 0.20   | 0.20              | 0.20   | 0.20                       | 0.20              | 0.15          |  |  |
| L-Thr                              | 0.18          | 0.18   | 0.18              | 0.18   | 0.18                       | 0.18              | 0.13          |  |  |
| L-Trp                              | 0.03          | 0.03   | 0.03              | 0.03   | 0.03                       | 0.03              | 0.01          |  |  |
| L-Val                              | 0.09          | 0.09   | 0.09              | 0.09   | 0.09                       | 0.09              | -             |  |  |
| Trace mineral premix <sup>§</sup>  | 0.09          | 0.09   | 0.09              | 0.09   | 0.09                       | 0.09              | 0.09          |  |  |
| Vitamin premix <sup>#</sup>        | 0.05          | 0.05   | 0.05              | 0.05   | 0.05                       | 0.05              | 0.05          |  |  |
| Choline chloride                   | 0.04          | 0.04   | 0.04              | 0.04   | 0.04                       | 0.04              | -             |  |  |
| Phytase <sup>&amp;</sup>           | -             | -      | 0.04              | -      | -                          | 0.04              | 0.02          |  |  |
| Zinc oxide                         | 0.27          | 0.27   | 0.27              | 0.27   | 0.27                       | 0.27              | -             |  |  |
| Selenium                           | 0.02          | 0.02   | 0.02              | 0.02   | 0.02                       | 0.02              | 0.02          |  |  |
| Total                              | 100.00        | 100.00 | 100.00            | 100.00 | 100.00                     | 100.00            | 100.00        |  |  |
| Calculated composition             |               |        |                   |        |                            |                   |               |  |  |
| Standardized ileal digestible      | <b>AA</b> . % |        |                   |        |                            |                   |               |  |  |
| Lys                                | 1 35          | 1 35   | 1 35              | 1 35   | 1 35                       | 1 35              | 1.27          |  |  |
| Ile:Lys                            | 61            | 61     | 61                | 61     | 60                         | 61                | 64            |  |  |
| Leu·Lys                            | 119           | 118    | 119               | 118    | 117                        | 118               | 127           |  |  |
| Met·Lys                            | 36            | 36     | 36                | 36     | 36                         | 36                | 35            |  |  |
| Met & Cys:Lys                      | 58            | 58     | 58                | 58     | 58                         | 58                | 59            |  |  |
| Thr·Lys                            | 65            | 65     | 65                | 65     | 65                         | 65                | 64            |  |  |
| Trn:Lys                            | 20            | 20     | 20                | 20     | 20                         | 20                | 20            |  |  |
| Val:Lys                            | 20<br>71      | 71     | 20<br>71          | 71     | 20<br>71                   | 71                | 20<br>68      |  |  |
| Total Lys %                        | 1 49          | 1 49   | 1 49              | 1 49   | 1 49                       | 1 49              | 1 42          |  |  |
| CP %                               | 20.68         | 20.66  | 20.70             | 20.61  | 20.59                      | 20.63             | 20.65         |  |  |
| NE kcal/kg                         | 2 535         | 2 535  | 2 535             | 2 536  | 2 535                      | 2 535             | 2 480         |  |  |
| $C_a$ no phytase %                 | 0.58          | 0.58   | 0.46              | 1.03   | 1.03                       | 0.91              | 0.76          |  |  |
| $C_{a}$ with phytase $\frac{1}{2}$ | 0.58          | 0.58   | 0.58              | 1.03   | 1.03                       | 1.03              | 0.76          |  |  |
| STTD P no phytase %                | 0.33          | 0.45   | 0.33              | 0.33   | 0.45                       | 0.33              | 0.00          |  |  |
| STTD P with phytase %              | 0.33          | 0.45   | 0.45              | 0.33   | 0.45                       | 0.45              | 0.37          |  |  |
| Total <b>P</b> %                   | 0.54          | 0.45   | 0.55              | 0.54   | 0.45                       | 0.43              | 0.47          |  |  |
| Analyzed composition %             | 0.54          | 0.00   | 0.55              | 0.54   | 0.00                       | 0.54              | 0.02          |  |  |
| DM                                 | 80.04         | 90.30  | 00.73             | 00.02  | 90.60                      | 00.33             | 88 76         |  |  |
| CP                                 | 22.05         | 20.50  | 20.75             | 20.92  | 21 45                      | 21 10             | 21.80         |  |  |
| Eat                                | 23.03<br>A 75 | 4 50   | 4 25              | 4 90   | 21. <del>4</del> 3<br>A 05 | A 45              | 21.0U<br>1.15 |  |  |
| r at<br>Ca <sup>§</sup>            | 4.75          | 4.50   | 4.23              | +.00   | +.75                       | 1.12              | 4.43          |  |  |
| Ca<br>D§                           | 0.05          | 0.03   | 0.54              | 1.32   | 0.76                       | 1.13              | 0.73          |  |  |
| 1                                  | 0.09          | 0.70   | 0.04              | 0.05   | 0.70                       | 0.02              | 0.09          |  |  |

\*Phase 2 diets were fed from d 14 to 28 and phase 3 diet were fed from d 28 to 42.

<sup>†</sup>Phytase was added to diets at the level of 1,000 phytase units with assumed release value of 0.12% for Ca and STTD P.

<sup>‡</sup>Hamlet Protein, Inc., Findlay, OH.

<sup>8</sup>Provided per kg of premix: 29.6 g Mn from manganese oxide, 104 g Fe from iron sulfate, 112 g Zn from zinc sulfate, 16 g Cu from copper sulfate, 1,600 mg I from calcium iodate.

<sup>#</sup>Provided per kg of premix: 28,659,800 IU vitamin A, 4,409,200 IU vitamin D<sub>3</sub>, 105,821 IU vitamin E, 801,665 mg vitamin K, 15,423 mg ribo-flavin, 66,138 mg pantothenic acid, 110,230 mg niacin, 79 mg vitamin  $B_{12}$ , 4,409 mg folic acid, 44 mg thiamin, 44 mg pyridoxine, and 4.4 mg biotin.

<sup>&</sup>Ronozyme HiPhos 2500 (DSM Nutritional Products, Inc., Parsippany, NJ).

<sup>s</sup>Averaged across analyzed values from Ward Laboratories, Inc., Cumberland Valley Analytical Services Inc., and Midwest Laboratories.

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| Table 4. Diet formulation, | phase 1 | (Exp. 2; | as-fed b | oasis) |
|----------------------------|---------|----------|----------|--------|
|----------------------------|---------|----------|----------|--------|

| Item                              |        | Phase 1 (d 0 to 10) |        |        |              |        |  |  |  |
|-----------------------------------|--------|---------------------|--------|--------|--------------|--------|--|--|--|
| STTD P:                           |        | NRC (0.45%)         |        | >N]    | >NRC (0.56%) |        |  |  |  |
| <u>Ca, %:</u>                     | 0.65   | 0.90                | 1.20   | 0.65   | 0.90         | 1.20   |  |  |  |
| Ingredients, %                    |        |                     |        |        |              |        |  |  |  |
| Corn                              | 46.66  | 45.54               | 44.14  | 46.04  | 44.92        | 43.53  |  |  |  |
| Soybean meal                      | 21.33  | 21.40               | 21.50  | 21.38  | 21.45        | 21.55  |  |  |  |
| HP 300*                           | 3.75   | 3.75                | 3.75   | 3.75   | 3.75         | 3.75   |  |  |  |
| Fish meal                         | 2.50   | 2.50                | 2.50   | 2.50   | 2.50         | 2.50   |  |  |  |
| Spray-dried whey                  | 20.00  | 20.00               | 20.00  | 20.00  | 20.00        | 20.00  |  |  |  |
| Beef tallow                       | 2.60   | 3.00                | 3.50   | 2.80   | 3.20         | 3.70   |  |  |  |
| Monocalcium P (21% P)             | 0.63   | 0.63                | 0.63   | 1.25   | 1.25         | 1.25   |  |  |  |
| Limestone                         | 0.30   | 0.95                | 1.75   | 0.04   | 0.70         | 1.49   |  |  |  |
| Salt                              | 0.50   | 0.50                | 0.50   | 0.50   | 0.50         | 0.50   |  |  |  |
| L-Lys HCl                         | 0.48   | 0.48                | 0.48   | 0.48   | 0.48         | 0.48   |  |  |  |
| DL-Met                            | 0.24   | 0.24                | 0.24   | 0.24   | 0.24         | 0.24   |  |  |  |
| L-Thr                             | 0.21   | 0.21                | 0.21   | 0.21   | 0.21         | 0.21   |  |  |  |
| L-Trp                             | 0.03   | 0.03                | 0.03   | 0.03   | 0.03         | 0.03   |  |  |  |
| L-Val                             | 0.15   | 0.15                | 0.15   | 0.15   | 0.15         | 0.15   |  |  |  |
| Trace mineral premix <sup>†</sup> | 0.09   | 0.09                | 0.09   | 0.09   | 0.09         | 0.09   |  |  |  |
| Vitamin premix <sup>‡</sup>       | 0.05   | 0.05                | 0.05   | 0.05   | 0.05         | 0.05   |  |  |  |
| Vitamin E (20,000 IU)             | 0.05   | 0.05                | 0.05   | 0.05   | 0.05         | 0.05   |  |  |  |
| Choline chloride                  | 0.04   | 0.04                | 0.04   | 0.04   | 0.04         | 0.04   |  |  |  |
| Zinc oxide                        | 0.39   | 0.39                | 0.39   | 0.39   | 0.39         | 0.39   |  |  |  |
| Selenium premix                   | 0.02   | 0.02                | 0.02   | 0.02   | 0.02         | 0.02   |  |  |  |
| Total                             | 100.00 | 100.00              | 100.00 | 100.00 | 100.00       | 100.00 |  |  |  |
| Calculated composition            |        |                     |        |        |              |        |  |  |  |
| Standardized ileal digestible A   | A, %   |                     |        |        |              |        |  |  |  |
| Lys                               | 1.40   | 1.40                | 1.40   | 1.40   | 1.40         | 1.40   |  |  |  |
| Ile:Lys                           | 55     | 55                  | 55     | 55     | 55           | 55     |  |  |  |
| Leu:Lys                           | 109    | 108                 | 108    | 109    | 108          | 107    |  |  |  |
| Met:Lys                           | 38     | 38                  | 38     | 38     | 38           | 38     |  |  |  |
| Met & Cys:Lys                     | 58     | 58                  | 57     | 58     | 57           | 57     |  |  |  |
| Thr:Lys                           | 64     | 63                  | 63     | 64     | 63           | 63     |  |  |  |
| Trp:Lys                           | 18     | 18                  | 18     | 18     | 18           | 18     |  |  |  |
| Val:Lys                           | 70     | 70                  | 70     | 70     | 70           | 70     |  |  |  |
| Total Lys, %                      | 1.53   | 1.53                | 1.53   | 1.53   | 1.53         | 1.53   |  |  |  |
| СР, %                             | 20.89  | 20.83               | 20.76  | 20.86  | 20.80        | 20.73  |  |  |  |
| NE, kcal/kg                       | 2,606  | 2,606               | 2,606  | 2,606  | 2,606        | 2,606  |  |  |  |
| Ca, %                             | 0.65   | 0.90                | 1.20   | 0.65   | 0.90         | 1.20   |  |  |  |
| STTD Ca <sup>§</sup> , %          | 0.51   | 0.67                | 0.87   | 0.53   | 0.69         | 0.88   |  |  |  |
| P, %                              | 0.66   | 0.65                | 0.65   | 0.78   | 0.78         | 0.77   |  |  |  |
| STTD P, %                         | 0.45   | 0.45                | 0.45   | 0.56   | 0.56         | 0.56   |  |  |  |
| Available P <sup>#</sup> , %      | 0.42   | 0.42                | 0.42   | 0.55   | 0.55         | 0.55   |  |  |  |
| Analyzed composition, %           |        |                     |        |        |              |        |  |  |  |
| DM                                | 90.42  | 90.09               | 90.91  | 89.84  | 90.15        | 89.82  |  |  |  |
| СР                                | 21.30  | 21.00               | 20.90  | 21.10  | 20.90        | 21.10  |  |  |  |
| Fat                               | 5.10   | 5.30                | 6.00   | 5.10   | 5.90         | 6.00   |  |  |  |
| Ca&                               | 0.66   | 0.80                | 1.23   | 0.66   | 0.82         | 1.27   |  |  |  |
| P&                                | 0.64   | 0.66                | 0.66   | 0.78   | 0.80         | 0.73   |  |  |  |

\*Hamlet Protein, Inc., Findlay, OH.

<sup>†</sup>Provided per kg of premix: 29.6 g Mn from manganese oxide, 104 g Fe from iron sulfate, 112 g Zn from zinc sulfate, 16 g Cu from copper sulfate, 1,600 mg I from calcium iodate.

<sup>1</sup>Provided per kg of premix: 28,659,800 IU vitamin A, 4,409,200 IU vitamin D<sub>3</sub>, 105,821 IU vitamin E, 801,665 mg vitamin K, 15,423 mg riboflavin, 66,138 mg pantothenic acid, 110,230 mg niacin, 79 mg vitamin B<sub>12</sub>, 4,409 mg folic acid, 44 mg thiamin, 44 mg pyridoxine, and 4.4 mg biotin.

Standardized total tract digestibility coefficients for Ca content of feed ingredients were from Stein (2016).

\*Determined using availability coefficients from NRC (1998).

\*Averaged across analyzed values from Ward Laboratories, Inc., Cumberland Valley Analytical Services Inc., and Midwest Laboratories.

Table 5. Diet formulation, phases 2 and 3 (Exp. 2; as-fed basis)\*

| Item                              | Phase 2 |             |        |        |              |        |        |  |
|-----------------------------------|---------|-------------|--------|--------|--------------|--------|--------|--|
| STTD P:                           |         | NRC (0.40%) |        | _      | >NRC (0.52%) |        |        |  |
| Ca, %:                            | 0.65    | 0.90        | 1.20   | 0.65   | 0.90         | 1.20   | 0.77   |  |
| Ingredients, %                    |         |             |        |        |              |        |        |  |
| Corn                              | 57.76   | 56.63       | 55.23  | 57.01  | 55.87        | 54.50  | 62.60  |  |
| Soybean meal                      | 24.88   | 24.96       | 25.05  | 24.93  | 25.01        | 25.10  | 32.23  |  |
| Fish meal                         | 3.50    | 3.50        | 3.50   | 3.50   | 3.50         | 3.50   | -      |  |
| Spray-dried whey                  | 10.00   | 10.00       | 10.00  | 10.00  | 10.00        | 10.00  | -      |  |
| Beef tallow                       | 1.00    | 1.40        | 1.90   | 1.25   | 1.65         | 2.15   | 1.00   |  |
| Monocalcium P (21% P)             | 0.61    | 0.61        | 0.61   | 1.29   | 1.29         | 1.29   | 1.25   |  |
| Limestone                         | 0.38    | 1.03        | 1.83   | 0.09   | 0.75         | 1.53   | 1.10   |  |
| Salt                              | 0.60    | 0.60        | 0.60   | 0.60   | 0.60         | 0.60   | 0.60   |  |
| L-Lys HCl                         | 0.40    | 0.40        | 0.40   | 0.40   | 0.40         | 0.40   | 0.40   |  |
| DL-Met                            | 0.18    | 0.18        | 0.18   | 0.18   | 0.18         | 0.18   | 0.18   |  |
| L-Thr                             | 0.17    | 0.17        | 0.17   | 0.17   | 0.17         | 0.17   | 0.17   |  |
| L-Trp                             | 0.03    | 0.03        | 0.03   | 0.03   | 0.03         | 0.03   | -      |  |
| L-Val                             | 0.10    | 0.10        | 0.10   | 0.10   | 0.10         | 0.10   | 0.07   |  |
| Trace mineral premix <sup>†</sup> | 0.09    | 0.09        | 0.09   | 0.09   | 0.09         | 0.09   | 0.09   |  |
| Vitamin premix <sup>‡</sup>       | 0.05    | 0.05        | 0.05   | 0.05   | 0.05         | 0.05   | 0.05   |  |
| Zinc oxide                        | 0.25    | 0.25        | 0.25   | 0.25   | 0.25         | 0.25   | 0.25   |  |
| Selenium premix                   | 0.02    | 0.02        | 0.02   | 0.02   | 0.02         | 0.02   | 0.02   |  |
| Total                             | 100.00  | 100.00      | 100.00 | 100.00 | 100.00       | 100.00 | 100.00 |  |
| Calculated composition            |         |             |        |        |              |        |        |  |
| Standardized ileal digestible     | AA, %   |             |        |        |              |        |        |  |
| Lys                               | 1.30    | 1.30        | 1.30   | 1.30   | 1.30         | 1.30   | 1.28   |  |
| Ile:Lys                           | 57      | 57          | 57     | 57     | 57           | 57     | 59     |  |
| Leu:Lys                           | 116     | 116         | 115    | 116    | 116          | 115    | 121    |  |
| Met:Lys                           | 37      | 37          | 36     | 37     | 37           | 36     | 36     |  |
| Met & Cys:Lys                     | 58      | 57          | 57     | 57     | 57           | 57     | 58     |  |
| Thr:Lys                           | 63      | 63          | 63     | 63     | 63           | 63     | 63     |  |
| Trp:Lys                           | 19      | 19          | 19     | 19     | 19           | 19     | 17     |  |
| Val:Lys                           | 70      | 69          | 69     | 69     | 69           | 69     | 69     |  |
| Total Lys, %                      | 1.44    | 1.44        | 1.44   | 1.44   | 1.44         | 1.44   | 1.43   |  |
| СР, %                             | 20.70   | 20.64       | 20.58  | 20.66  | 20.61        | 20.54  | 21.19  |  |
| NE, kcal/kg                       | 2,518   | 2,518       | 2,518  | 2,518  | 2,518        | 2,518  | 2,445  |  |
| Ca, %                             | 0.65    | 0.90        | 1.20   | 0.65   | 0.90         | 1.20   | 0.77   |  |
| STTD Ca <sup>§</sup> , %          | 0.49    | 0.65        | 0.85   | 0.51   | 0.67         | 0.86   | 0.54   |  |
| P, %                              | 0.62    | 0.62        | 0.62   | 0.76   | 0.75         | 0.75   | 0.61   |  |
| STTD P, %                         | 0.40    | 0.40        | 0.40   | 0.52   | 0.52         | 0.52   | 0.37   |  |
| Available P <sup>#</sup> , %      | 0.36    | 0.36        | 0.36   | 0.50   | 0.50         | 0.50   | 0.32   |  |
| Analyzed composition, %           |         |             |        |        |              |        |        |  |
| DM                                | 89.96   | 89.24       | 89.83  | 89.61  | 90.05        | 89.35  | 88.60  |  |
| СР                                | 21.20   | 21.10       | 21.30  | 21.10  | 21.70        | 21.20  | 21.30  |  |
| Fat                               | 4.00    | 4.20        | 4.50   | 4.20   | 4.50         | 4.90   | 4.00   |  |
| Ca&                               | 0.73    | 0.97        | 1.33   | 0.72   | 0.93         | 1.24   | 0.76   |  |
| P&                                | 0.64    | 0.63        | 0.66   | 0.79   | 0.81         | 0.76   | 0.65   |  |

\*Phase 2 diets were fed from d 10 to 24 and phase 3 diet were fed from d 24 to 45.

<sup>†</sup>Provided per kg of premix: 29.6 g Mn from manganese oxide, 104 g Fe from iron sulfate, 112 g Zn from zinc sulfate, 16 g Cu from copper sulfate, 1,600 mg I from calcium iodate.

<sup>t</sup>Provided per kg of premix: 28,659,800 IU vitamin A, 4,409,200 IU vitamin D<sub>3</sub>, 105,821 IU vitamin E, 801,665 mg vitamin K, 15,423 mg riboflavin, 66,138 mg pantothenic acid, 110,230 mg niacin, 79 mg vitamin B<sub>12</sub>, 4,409 mg folic acid, 44 mg thiamin, 44 mg pyridoxine, and 4.4 mg biotin.

STTD coefficients for Ca content of feed ingredients were from Stein (2016).

<sup>#</sup>Determined using availability coefficients from NRC (1998).

<sup>&</sup>Averaged across analyzed values from Ward Laboratories, Inc., Cumberland Valley Analytical Services Inc., and Midwest Laboratories.

| Item                       |                     |                     | Treat               | ment              |                    |                     |       | Pro           | bability, P | <     |
|----------------------------|---------------------|---------------------|---------------------|-------------------|--------------------|---------------------|-------|---------------|-------------|-------|
| Ca, %:                     | 0.58                | 0.58                | 0.58 <sup>†</sup>   | 1.03              | 1.03               | 1.03 <sup>+</sup>   | _     | _             |             |       |
| STTD P, no phytase, %:     | 0.33                | 0.45                | 0.33                | 0.33              | 0.45               | 0.33                | SEM   | $Ca \times P$ | Ca          | Р     |
| STTD P, with phytase, %:   | -                   | -                   | 0.45                | -                 | -                  | 0.45                |       |               |             |       |
| BW, kg                     |                     |                     |                     |                   |                    |                     |       |               |             |       |
| d 0                        | 6.1                 | 6.1                 | 6.1                 | 6.1               | 6.1                | 6.1                 | 0.06  | 0.773         | 0.609       | 0.208 |
| d 14                       | 8.9 <sup>bc</sup>   | 9.1 <sup>b</sup>    | 10.0 <sup>a</sup>   | 8.6°              | 9.3 <sup>b</sup>   | 10.0 <sup>a</sup>   | 0.13  | 0.023         | 0.439       | 0.001 |
| d 28                       | 16.4°               | 16.4°               | 17.8 <sup>a</sup>   | 14.9 <sup>d</sup> | 16.8 <sup>bc</sup> | 17.3 <sup>ab</sup>  | 0.21  | 0.001         | 0.003       | 0.001 |
| d 42                       | 27.6 <sup>ab</sup>  | 27.6 <sup>ab</sup>  | 28.6 <sup>a</sup>   | 26.7 <sup>b</sup> | 28.2ª              | 28.7ª               | 0.32  | 0.034         | 0.853       | 0.001 |
| Phase 1 (d 0 to 14)        |                     |                     |                     |                   |                    |                     |       |               |             |       |
| ADG, g                     | 204 <sup>bc</sup>   | 216 <sup>b</sup>    | 283 <sup>a</sup>    | 179°              | 231 <sup>b</sup>   | 279 <sup>a</sup>    | 7.2   | 0.019         | 0.393       | 0.001 |
| ADFI, g                    | 272 <sup>ь</sup>    | 272 <sup>ь</sup>    | 338 <sup>a</sup>    | 269 <sup>b</sup>  | 288 <sup>b</sup>   | 334 <sup>a</sup>    | 7.2   | 0.241         | 0.594       | 0.001 |
| G:F, g/kg                  | 749 <sup>ь</sup>    | 794 <sup>ab</sup>   | 835 <sup>a</sup>    | 665°              | 802 <sup>a</sup>   | 836 <sup>a</sup>    | 12.7  | 0.001         | 0.015       | 0.001 |
| Phase 2 (d 14 to 28)       |                     |                     |                     |                   |                    |                     |       |               |             |       |
| ADG, g                     | 534 <sup>a</sup>    | 522 <sup>a</sup>    | 545ª                | 451 <sup>b</sup>  | 535ª               | 522 <sup>a</sup>    | 10.5  | 0.001         | 0.001       | 0.001 |
| ADFI, g                    | 725°                | 741 <sup>bc</sup>   | 780 <sup>ab</sup>   | 712°              | 780 <sup>ab</sup>  | 789 <sup>a</sup>    | 10.9  | 0.050         | 0.173       | 0.001 |
| G:F, g/kg                  | 737 <sup>a</sup>    | 704 <sup>ab</sup>   | 699 <sup>ab</sup>   | 633 <sup>d</sup>  | 686 <sup>bc</sup>  | 661 <sup>cd</sup>   | 8.0   | 0.001         | 0.001       | 0.165 |
| Treatment (d 0 to 28)      |                     |                     |                     |                   |                    |                     |       |               |             |       |
| ADG, g                     | 365°                | 365°                | 411ª                | 312 <sup>d</sup>  | 379 <sup>bc</sup>  | 398 <sup>ab</sup>   | 6.6   | 0.001         | 0.002       | 0.001 |
| ADFI, g                    | 493°                | 501 <sup>bc</sup>   | 554ª                | 485°              | 528 <sup>ab</sup>  | 556 <sup>a</sup>    | 7.1   | 0.042         | 0.217       | 0.001 |
| G:F, g/kg                  | 740 <sup>a</sup>    | 729 <sup>a</sup>    | 742ª                | 642 <sup>b</sup>  | 718 <sup>a</sup>   | 715 <sup>a</sup>    | 6.2   | 0.001         | 0.001       | 0.001 |
| Posttreatment (d 28 to 42) |                     |                     |                     |                   |                    |                     |       |               |             |       |
| ADG, g                     | 800 <sup>ab</sup>   | 798 <sup>ab</sup>   | 774 <sup>b</sup>    | 842ª              | 815 <sup>ab</sup>  | 816 <sup>ab</sup>   | 11.8  | 0.428         | 0.001       | 0.068 |
| ADFI, g                    | 1056°               | 1066 <sup>ab</sup>  | 1073 <sup>ab</sup>  | 1042°             | 1121ª              | 1093 <sup>ab</sup>  | 14.7  | 0.063         | 0.093       | 0.007 |
| G:F, g/kg                  | 757 <sup>b</sup>    | 749 <sup>bc</sup>   | 722°                | 809 <sup>a</sup>  | 727 <sup>bc</sup>  | 747 <sup>bc</sup>   | 9.4   | 0.001         | 0.010       | 0.001 |
| d 0 to 42                  |                     |                     |                     |                   |                    |                     |       |               |             |       |
| ADG, g                     | 502 <sup>ab</sup>   | 502 <sup>ab</sup>   | 526 <sup>a</sup>    | 479 <sup>b</sup>  | 517ª               | 530 <sup>a</sup>    | 6.9   | 0.020         | 0.805       | 0.001 |
| ADFI, g                    | 701°                | 706 <sup>bc</sup>   | 742 <sup>ab</sup>   | 692°              | 742 <sup>ab</sup>  | 750 <sup>a</sup>    | 9.0   | 0.044         | 0.130       | 0.001 |
| G:F, g/kg                  | 715 <sup>a</sup>    | 710 <sup>ab</sup>   | 708 <sup>ab</sup>   | 692 <sup>b</sup>  | 697 <sup>b</sup>   | 707 <sup>ab</sup>   | 4.5   | 0.055         | 0.001       | 0.616 |
| Bone ash <sup>‡</sup> , %  | 44.11 <sup>bc</sup> | 45.62 <sup>ab</sup> | 45.75 <sup>ab</sup> | 42.63°            | 47.95ª             | 45.50 <sup>ab</sup> | 0.611 | 0.007         | 0.692       | 0.001 |

**Table 6.** Effects of Ca and P concentrations on growth performance of nursery pigs (Exp. 1)\*

\*A total of 720 mixed gender pigs (PIC 280 × Camborough, Genus PIC, Hendersonville, TN) with initial BW of  $6.1 \pm 0.98$  kg were used in a 42-d growth trial with 10 pigs per pen and 12 replications (pen) per treatment. One pen from 0.58% Ca + 0.45% STTD P treatment encountered issues with feeder allowance and had restricted feed intake; therefore, data from this pen were excluded from all the analyses.

<sup>†</sup>Phytase (Ronozyme HiPhos 2500, DSM Nutritional Products, Inc., Parsippany, NJ) was added to diets at the level of 1,000 phytase units with assumed release value of 0.12% for Ca and STTD P.

<sup>‡</sup>Radius samples collected from 1 median-weight gilt from each pen on d 21.

<sup>abcd</sup>Means with different superscripts within a row differ (P < 0.05).

phytase, but this effect was only observed when high Ca was fed. For ADFI, when diets contained low Ca concentration, feeding 0.45% STTD P with phytase resulted in greater (P = 0.018) ADFI than feeding 0.33% STTD P diet, with that of pigs fed 0.45% STTD P without phytase intermediate. When high Ca was fed, ADFI of pigs fed 0.45% STTD P with or without phytase was greater (P < 0.01) than those fed 0.33% STTD P. Dietary STTD P level did not affect overall G:F regardless of Ca concentration; however, G:F was decreased (P = 0.005) by feeding 1.03% Ca compared with feeding 0.58% Ca when diets contained 0.33% STTD P. This Ca effect was not observed when diets contained 0.45% STTD P with or without phytase. Similarly, final BW of pigs fed 0.33% STTD P was decreased

(P < 0.01) relative to pigs fed 0.45% STTD P with or without phytase when high Ca was fed with no P response with low-dietary Ca concentration.

Pigs fed 0.33% STTD P had decreased (P < 0.05) percentage bone ash compared with those fed 0.45% STTD P with or without phytase when high Ca was added to diets, but this P effect was not observed among treatments with low Ca concentration (Ca × P interaction, P = 0.007).

## **Experiment 2**

During phase 1 (d 0 to 10), no evidence of Ca  $\times$  P interactions were observed for any growth criteria (*P* > 0.38; Table 7). Calcium and STTD P concentrations did not affect ADG or d 10 BW. However,

| Item            | em Treatment     |                  |                  |                  |                   |                  |      |              | Probability, <i>P</i> < |        |        |           |
|-----------------|------------------|------------------|------------------|------------------|-------------------|------------------|------|--------------|-------------------------|--------|--------|-----------|
| STTD P:         |                  | NRC <sup>†</sup> |                  |                  | >NRC <sup>‡</sup> |                  |      |              | Main                    | effect | Ca     | l         |
| Ca, %:          | 0.65             | 0.90             | 1.20             | 0.65             | 0.90              | 1.20             | SEM  | Ca × P       | Ca                      | Р      | Linear | Quadratic |
| BW, kg          |                  |                  |                  |                  |                   |                  |      |              |                         |        |        |           |
| d 0             | 6.0              | 6.0              | 6.0              | 6.0              | 6.0               | 6.0              | 0.01 | 0.968        | 0.989                   | 0.758  | 0.897  | 0.947     |
| d 10            | 6.8              | 6.8              | 6.8              | 6.8              | 6.7               | 6.7              | 0.06 | 0.833        | 0.502                   | 0.129  | 0.410  | 0.406     |
| d 24            | 11.5             | 11.5             | 10.7             | 11.7             | 11.5              | 11.4             | 0.19 | 0.181        | 0.018                   | 0.096  | 0.006  | 0.560     |
| d 45            | 25.2             | 25.5             | 24.8             | 25.3             | 25.3              | 25.2             | 0.36 | 0.644        | 0.467                   | 0.756  | 0.381  | 0.388     |
| Phase 1 (d 0 to | o 10)            |                  |                  |                  |                   |                  |      |              |                         |        |        |           |
| ADG, g          | 77               | 73               | 75               | 73               | 64                | 65               | 5.9  | 0.879        | 0.507                   | 0.138  | 0.418  | 0.405     |
| ADFI, g         | 108              | 107              | 115              | 101              | 102               | 117              | 4.4  | 0.551        | 0.022                   | 0.433  | 0.014  | 0.188     |
| G:F, g/kg       | 709              | 680              | 651              | 724              | 624               | 555              | 40.3 | 0.386        | 0.029                   | 0.173  | 0.009  | 0.709     |
| Phase 2 (d 10 t | to 24)           |                  |                  |                  |                   |                  |      |              |                         |        |        |           |
| ADG, g          | 339 <sup>a</sup> | 335 <sup>a</sup> | 281 <sup>b</sup> | 353 <sup>a</sup> | 341ª              | 337 <sup>a</sup> | 11.6 | $0.088^{\$}$ | 0.008                   | 0.011  | 0.003  | 0.371     |
| ADFI, g         | 441              | 457              | 444              | 459              | 441               | 435              | 13.5 | 0.406        | 0.699                   | 0.860  | 0.434  | 0.760     |
| G:F, g/kg       | 769 <sup>a</sup> | 732 <sup>a</sup> | 637 <sup>b</sup> | 767 <sup>a</sup> | 774 <sup>a</sup>  | 773 <sup>a</sup> | 16.3 | 0.001#       | 0.002                   | 0.001  | 0.001  | 0.336     |
| Treatment (d (  | ) to 24)         |                  |                  |                  |                   |                  |      |              |                         |        |        |           |
| ADG, g          | 230              | 226              | 195              | 236              | 226               | 224              | 8.1  | 0.203        | 0.020                   | 0.084  | 0.006  | 0.622     |
| ADFI, g         | 302              | 311              | 307              | 310              | 300               | 303              | 9.0  | 0.559        | 0.988                   | 0.753  | 0.876  | 0.994     |
| G:F, g/kg       | 760 <sup>a</sup> | 725 <sup>a</sup> | 639 <sup>b</sup> | 761ª             | 753ª              | 738 <sup>a</sup> | 16.3 | 0.015#       | 0.001                   | 0.003  | 0.001  | 0.439     |
| Posttreatment   | (d 24 to 45      | 5)               |                  |                  |                   |                  |      |              |                         |        |        |           |
| ADG, g          | 650              | 669              | 660              | 644              | 658               | 656              | 10.4 | 0.944        | 0.294                   | 0.395  | 0.337  | 0.216     |
| ADFI, g         | 978              | 977              | 944              | 976              | 962               | 961              | 17.9 | 0.684        | 0.383                   | 0.990  | 0.175  | 0.816     |
| G:F, g/kg       | 665              | 685              | 699              | 660              | 684               | 685              | 9.0  | 0.747        | 0.008                   | 0.354  | 0.003  | 0.269     |
| d 0 to 45       |                  |                  |                  |                  |                   |                  |      |              |                         |        |        |           |
| ADG, g          | 426              | 431              | 411              | 425              | 427               | 425              | 8.3  | 0.532        | 0.424                   | 0.622  | 0.360  | 0.349     |
| ADFI, g         | 618              | 619              | 603              | 618              | 609               | 610              | 11.4 | 0.758        | 0.588                   | 0.939  | 0.310  | 0.908     |
| G:F, g/kg       | 690              | 696              | 682              | 687              | 702               | 699              | 8.2  | 0.510        | 0.399                   | 0.322  | 0.906  | 0.180     |

Table 7. Effects of Ca and P concentrations on growth performance of nursery pigs (Exp. 2)\*

\*A total of 360 barrows and gilts (PIC 280  $\times$  Camborough, Genus PIC, Hendersonville, TN) with initial BW of 6.0  $\pm$  1.08 kg were used in a 45-d trial with 10 pigs per pen and six replications (pen) per treatment.

<sup>††</sup>NRC = STTD P levels formulated to meet NRC (2012) requirement estimates (0.45% for phase 1 and 0.40% for phase 2).

\*>NRC = STTD P levels formulated to exceed NRC (2012) requirement estimates (0.56% for phase 1 and 0.52% for phase 2).

<sup>§</sup>Linear Ca × P interaction: P = 0.070; quadratic Ca × P interaction: P = 0.196.

<sup>*H*</sup>Linear Ca × P interaction: P < 0.01; quadratic Ca × P interaction: P > 0.10.

<sup>ab</sup>Means with different superscripts within a row differ (P < 0.05).

10

increasing Ca increased (linear, P = 0.014) ADFI but decreased (linear, P = 0.009) G:F.

During phase 2 (d 10 to 24), a marginal Ca × P interaction was observed for ADG (P = 0.088) and a significant interaction for G:F (P = 0.001), but not for ADFI or BW. Pigs fed 1.20% Ca had decreased (P < 0.05) ADG and G:F compared with those fed 0.65% and 0.90% Ca when diets contained NRC STTD P; however, this detrimental effect of high Ca was not observed in pigs fed >NRC STTD P. Average daily feed intake was not affected by dietary Ca or STTD P. Day 24 BW was decreased (linear, P = 0.006) by increasing Ca regardless of the STTD P concentration in diets. Feeding >NRC STTD P resulted in a marginally greater (P = 0.096) d 24 BW than those fed NRC STTD P.

When combining the treatment periods (d 0 to 24), no Ca  $\times$  P interactions were observed for

ADG and ADFI. Increasing Ca decreased (linear, P = 0.006) ADG but had no evidence for an effect on ADFI. Similarly, feeding >NRC STTD P marginally increased (P = 0.084) ADG but had no evidence for an effect on ADFI, compared with pigs fed NRC STTD P. Concentrations of Ca and STTD P had an interactive effect on G:F (P = 0.015). When diets contained NRC STTD P, pigs fed 1.20% Ca had poorer (P < 0.05) G:F than those fed 0.65% and 0.90% Ca; however, when >NRC STTD P was fed, G:F was not affected by dietary Ca concentration.

During the posttreatment period from d 24 to 45, all pigs received a common phase 3 diet. No interactive or main effects of Ca and STTD P concentrations were observed for ADG, ADFI, or final BW. However, pigs previously fed increasing dietary Ca had improved (linear, P = 0.003) G:F regardless

of the STTD P content previously fed in phase 1 and 2 diets. As a result of this compensatory gain, overall (d 0 to 45) growth responses were not affected by the Ca and P concentrations fed during phases 1 and 2.

#### DISCUSSION

In high Ca diets, free Ca binds with P in the chyme to form insoluble salts, resulting in decreased digestion and absorption of dietary P (Heaney and Nordin, 2002). As an example, Stein et al. (2011) reported a linear reduction of apparent total tract digestibility of P from 56.9% to 46.2% when dietary Ca increased from 0.33% to 1.04% in growing pig. Therefore, it has been widely established that excess Ca may negatively affect pig growth performance depending on the level of P in diets (Reinhart and Mahan, 1986; Liu et al., 1998; González-Vega et al., 2016). The total Ca and STTD P requirements estimated by NRC (2012) are 0.85% and 0.45%, respectively, for 5 to 7 kg (phase 1) pigs and 0.80% and 0.40%, respectively, for 7 to 11 kg (phase 2) pigs. In Exp. 1, we observed that feeding 1.03% total Ca decreased ADG, ADFI, and G:F when diets were deficient in STTD P (0.33%), but these detrimental effects of excess Ca were not observed when adequate P diets (0.45%) were fed. This observation is in agreement with a recent study in 100- to 130kg finishing pigs where excess Ca (total Ca:STTD P ratio greater than 2.2:1) in diets decreased ADG only when STTD P was at or below the NRC (2012)-estimated requirements (Merriman et al., 2017). Results from Exp. 2 suggest that increasing dietary Ca decreased G:F independent of STTD P in phase 1. However, during phase 2, the detrimental effects of high Ca on ADG and G:F were only observed in pigs fed NRC STTD P (0.40%) but not for pigs fed 0.52% STTD P. It is possible that 0.40%STTD P just met, or was marginally below, the requirement of pigs during phase 2, which resulted in a P deficiency when high Ca was added to the diets. This marginal deficiency in STTD P is also supported by the observation that feeding high levels of STTD P (>NRC) tended to improve ADG from d 0 to 24. Vier et al. (2017) also reported that NRC (2012) may underestimate STTD P requirements for optimal performance and economic return in 11 to 25 kg nursery pigs.

Reinhart and Mahan (1986) observed that when diets contained low P (0.05% below NRC), total Ca:total P ratios above 1.3:1 decreased growth performance of pigs in any production phase, whereas when high dietary P (0.10% above NRC) was provided, wide total Ca:total P ratio up to 2.0:1 could be fed without detrimental effects. In another study, Qian et al. (1996) observed improved growth performance of 9 to 23 kg pigs when total Ca:total P ratio was narrowed from 2.0:1 to 1.2:1 regardless of dietary P concentration (0.36% or 0.45% total P). In the present study, total Ca:total P ratios ranging from 0.8:1 to 1.6:1 were fed without reduction in growth performance, but decreased performance was observed when total Ca:total P ratio exceeded 1.9:1. Interestingly, during the common phases of both the experiments, pigs previously fed low STTD P and high Ca diets grew faster and were more efficient than pigs from other treatments, suggesting a compensatory gain effect in response to the increased P and reduced Ca concentrations in the phase 3 diet. However, in Exp.1, these pigs were not able to fully compensate for the negative effects of P deficiency when diets contained excess Ca. In contrast to the compensatory gain observed in our study, Gonzalo et al. (2017) studied the effects of P depletion and repletion on growing-finishing pig performance and observed that previous P deficiency decreased ADG and ADFI during the subsequent repletion period.

Supplementing phytase to low P diets alleviated the impact of P deficiency on growth performance, and the magnitude of improvement was greater in diets containing high Ca. This observation is expected because increasing STTD P above the requirement of pigs by adding phytase improves their tolerance to wide Ca:P ratio. Moreover, the diets that included phytase to achieve 0.45% STTD P also improved ADG and ADFI of pigs over the diets containing 0.45% STTD P from only inorganic source, and this phytase response was more evident during phase 1 of the experiment, when dietary P would have been more limiting than during phase 2. It is possible that the 0.12% release value suggested by the manufacturer for 1,000 FYT of phytase underestimated the true digestible P and Ca release, resulting in more Ca, P, or possibly other nutrients becoming available to the pig.

Dietary Ca concentration has also been reported to alter the releasing ability of phytase. Proposed mechanisms for a Ca-phytase interaction include: 1) formation of a Ca-phytate complex that reduces the solubility of phytate and its accessibility by phytase; 2) competition of Ca for active sites of the enzyme resulting in indirect repression of phytase activity; and 3) a high acid binding capacity of inorganic Ca sources may influence phytase activity depending on their pH activity spectrum (Selle et al., 2009). Qian et al. (1996) suggested that increasing total Ca:total P ratio between 1.2:1 and 2.0:1 in diets resulted in approximately 1.95% reduction in the efficacy of supplemental phytase for each 0.1 unit change in Ca:P ratio. However, the negative effects of high Ca on phytase activity were not observed in the present study. Feeding 1.03% total Ca to phytase-supplemented diets resulted in similar growth performance and percentage bone ash as those fed 0.58% Ca.

According to the Ca  $\times$  P interaction observed for bone ash concentration, increasing dietary Ca exacerbated the deficiency of P (feeding 0.33%) STTD P) for bone mineralization, compared with an improvement when diets contained adequate P (0.45% STTD P). This can be explained by the fact that a wider Ca:P ratio (about 2.2:1) is required to form hydroxyapatite-like compounds for bone development (Crenshaw, 2001). Similar observations were reported by Létourneau-Montminy et al. (2012) where increasing dietary Ca from 0.5 to 0.8%decreased P retention by 0.016% in pigs fed a diet containing 0.1% nonphytate P, while it increased P retention of pigs by 0.026% when diet contained 0.3% nonphytate P. Furthermore, the growth promoting effects of phytase were not observed for percentage bone ash. This observation is in contrast with the growth performance data, where it appeared that the P release by adding phytase was underestimated. Therefore, it is possible that the beneficial effect of phytase on growth performance was a result of liberating other nutrients in the diet.

In summary, our data suggest that feeding excess dietary Ca negatively affected growth performance and percentage bone ash of nursery pigs when diets are deficient in STTD P. The STTD P estimates by NRC (2012) met the requirement of nursery pigs when diets contain low Ca concentrations, but resulted in decreased growth performance when diets contained more than 0.90% Ca. Future research is in need to determine the optimal Ca:P ratio in early nursery diets. Moreover, adding inorganic P or phytase to P deficient diets improved pig performance and alleviated the negative impacts of high dietary Ca concentration on growth performance.

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