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A cooperative study on the standardized total-tract digestible phosphorus requirement of twenty-kilogram pigs¹

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North Central Coordinating Committee on Swine Nutrition^{3,4,5}

ABSTRACT: A cooperative study comprising growth performance, bone mineralization, and nutrient balance experiments was conducted at 11 stations to determine the standardized total-tract digestible (STTD) P requirement of 20-kg pigs using broken-line regression analysis. Monocalcium phosphate and limestone were added to a corn-soybean meal-based diet at the expense of cornstarch to establish 6 concentrations of STTD P from 1.54 to 5.15 g/kg in increments of 0.62 g/kg at a constant Ca:total P of 1.52:1.0. Diets were fed to 936 pigs (average initial BW of 19 kg) in 240 pens for 20 replicate pens of barrows and 20 replicate pens of gilts per diet. As STTD P increased from 1.54 to 5.15 g/kg of the diet for d 0 to 14, 14 to 28, and 0 to 28, the ADG, ADFI, and G:F increased ($P < 0.01$). Barrows gained and ate more ($P < 0.05$) than gilts during d 14 to 28 and 0 to 28. There was no interaction between sex and STTD P concentration for any of the growth performance response criteria. There were both linear and quadratic increases ($P < 0.05$) in mineral density and content of ash, Ca, and P in the femur expressed as a percentage of dry, fat-free metacarpal as dietary STTD P increased. Furthermore, the maximum load of the femur and min-

eral density and content and maximum load as well as the Ca and P expressed as a percentage of metacarpal ash linearly increased ($P < 0.01$) with increasing dietary concentrations of STTD P. There were both linear and quadratic increases ($P < 0.01$) in apparent digestibility and retention of P with increasing concentrations of STTD P in the diets. Digestibility and retention of Ca linearly ($P < 0.01$) increased with increasing dietary concentrations of STTD P. Breakpoints determined from nonlinear broken-line regression analyses revealed estimates of 4.20 ± 0.102 , 3.20 ± 0.036 , or 3.87 ± 0.090 g/kg for ADG during d 0 to 14, 14 to 28, or 0 to 28, respectively. Corresponding estimates using G:F as the response criterion were 4.34 ± 0.146 , 3.38 ± 0.139 , or 4.08 ± 0.195 g/kg. When mineralization of the femur was used as criteria of response, estimates of STTD P requirement were 4.28, 4.28, or 4.34, g/kg for mineral density, mineral content, or maximum load, respectively. Using mineralization of the metacarpal as criteria of response, estimates of STTD P requirement ranged from 3.5 to 5.0 g/kg depending on the metacarpal response criteria. The study provided empirical estimates of STTD P requirements of 20- to 40-kg pigs.

Key words: phosphorus, pigs, requirement, standardized total-tract digestible

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Table 1. Participating stations and their contributions to the growth performance study

Research station	No. of replicate pens	No. of pigs per pen	Total no. of pigs	Initial BW, kg	Final BW, kg
North Carolina State University (Raleigh, NC)	4	4	96	18.2	38.8
Oklahoma State University (Stillwater, OK)	4	4	96	18.0	36.8
Purdue University (West Lafayette, IN)	4	4	96	19.5	42.2
University of Arkansas (Fayetteville, AK)	4	6	144	19.6	38.6
University of Georgia (Athens, GA)	4	3	72	18.1	42.0
University of Kentucky (Lexington, KY)	4	3	72	19.9	40.9
University of Missouri (Columbia, MO)	4	3	72	18.8	37.6
University of Nebraska (Lincoln, NE)	4	4	96	19.2	40.6
University of Wisconsin (Madison, WI)	4	4	96	19.4	43.6
Virginia Polytechnic Institute and State University (Sufflok, VA)	4	4	96	19.3	41.3
Total	40		936		
Average				19.0	40.2
USDA-ARS, Ames, IA					Chemical analyses of all samples

INTRODUCTION

Required for a variety of diverse biological functions, P is the second most abundant mineral element in the body following Ca (Berndt and Kumar, 2009; NRC, 2012). The requirement of a pig for P can be estimated either by factorial or empirical approaches. The factorial approach is considered to be advantageous considering its broader application to various systems of production (Jongbloed and Everts, 1992; NRC 2012). Routine empirical experiments provide estimates of requirements reflective of the changes over time in swine genetics, feedstuffs, and environment. The NRC (2012) adopted the concept of standardized total-tract digestibility of P to describe P utilization in feed ingredients by pigs as well as requirements for P. In spite of the extensive literature evaluating P in growing pigs, a need for more empirical studies on the standardized total-tract digestible (STTD) P requirements of pigs was emphasized by the NRC (2012). The models used for estimating P requirement by the NRC (2012) require further empirical data to validate the model-derived digestible P requirement. In recent publications, Zhai and Adeola (2013a,b) reported the results of experiments designed to assess the digestible P requirement of pigs using 6 diets in each study, but the genetic variation and environmental conditions would represent only a limited portion of the commercial variation.

Because of the dearth of empirical data on STTD P requirements of pigs, we reasoned that a multiuniversity study should be used to generate data to estimate STTD P requirements. A collaborative study involving stations with varying genetics, environments, and management conditions would provide more robust empirical data for estimates of STTD P requirements. Therefore, the experiments reported in this communication were designed to determine the STTD P requirement of 20-kg pigs. The multiuniversity collaborative study used 6 dietary concentrations of STTD P fed to 20

replicate pens of barrows and 20 replicate pens of gilts per diet with a broad set of response criteria.

MATERIALS AND METHODS

This cooperative study comprised experiments on growth performance, mineralization of bones, and nutrient balance. Ten research stations listed in Table 1 conducted growth performance experiments involving a total of 468 crossbred barrows and 468 crossbred gilts, and 1 research station exclusively conducted all the chemical analyses. Each station contributed 2 replicate pens of barrows and 2 replicate pens of gilts with 3 to 6 pigs per pen, resulting in a total of 20 replicates of each sex for the growth performance experiment. The average initial BW was 19 kg (range from 18.0 to 19.9 kg), and the range of final BW was from 36.8 to 43.6 kg with an average of 40.2 kg (Table 1). In addition, 3 of the 10 stations used a total of 72 pigs (1 pig from each pen) for determination of the mineralization of the femur and the third and fourth metacarpals. Furthermore, nutrient balance studies were conducted at 2 of the 10 stations using 8 barrows per diet for a total of 48 pigs. The experimental protocol was approved by the Institutional Animal Care and Use Committee at each participating station.

Diets, Monocalcium Phosphate, and Limestone

Mash diets were prepared using locally procured ingredients, including vitamin, mineral, and selenium premixes, at each participating station with the exception of monocalcium phosphate (MCP) and limestone. Ingredient composition of the 6 diets used at each participating station and formulated to contain 0.99% standardized ileal digestible Lys and 19% CP is presented in Table 2. Each station stored adequate amounts of locally procured ingredients for the entire study. A common source of the same batch of MCP and limestone, the

analyzed compositions of which are presented in Table 3, were procured from Akey, Inc. (Brookville, OH) and shipped to all participating stations. Monocalcium phosphate was added to a corn–soybean meal–based diet at the expense of cornstarch to establish the 6 concentrations of STTD P, ranging from 1.54 to 5.15 g/kg of diet, in increments of 0.62 g/kg. Standardized total-tract digestibility of P values for corn, soybean meal, and MCP published by the NRC (2012) were used to derive formulated STTD P concentrations in the diets. Limestone was added, also at the expense of cornstarch, to maintain a constant Ca:total P at 1.52:1.0 among all diets (Table 2). Each station sent samples of the 6 diets, corn, and soybean meal to the research station that conducted all the chemical analyses.

Growth Performance

The experiment was conducted as a randomized complete block design with 6 dietary treatments (Table 2) and 2 replicate pens of barrows and 2 replicate pens of gilts per diet for each of the 10 stations participating in the performance study. Each station allotted pigs to treatments on the basis of initial BW and sex for the growth performance study. Pigs selected for the study at each station were fed a nutrient-adequate (especially Ca and P) diet for at least 2 wk before initiation of the growth performance study. Pigs and feeders were weighed on d 0, 14, and 28 to record and calculate ADG, ADFI, and G:F. Pigs had free access to feed and water throughout the 28-d study.

Femur and Third and Fourth Metacarpals

Three of 10 stations selected, on d 28 of the trial, 1 pig nearest to the mean pen BW from each pen to determine the mineralization of bone. Following stunning and exsanguination, both the right and left femurs were separated at the pelvis and knee joints, leaving articular cartilage on the femur surfaces intact. Individual femurs were placed in a zip-lock plastic bag with a permanent identification tag within the bag. Femurs were frozen and shipped frozen with gel ice packs to the University of Wisconsin (Madison, WI) for bone mineral content (BMC) and bone mineral density (BMD) analyses using dual-energy X-ray absorptiometry (DXA) and a mechanical test. Both right and left front feet were excised at the junction of carpals and radius and ulna, individually placed in a zip-lock plastic bag, frozen, and shipped frozen with gel ice packs to the University of Wisconsin for DXA scans. Following the mineral content and density analyses by DXA scans, the front feet were shipped to the University of Kentucky (Lexington, KY) for metacarpal bone strength, ash, Ca, and P analyses.

Table 2. Ingredient composition (%) of the 6 experimental diets formulated to contain 1.54, 2.26, 2.98, 3.70, 4.43, or 5.15 g standardized total-tract digestible (STTD) P/kg on an as-fed basis

Ingredient	STTD P, g/kg					
	1.54	2.26	2.98	3.70	4.43	5.15
Corn	64.0	64.0	64.0	64.0	64.0	64.0
Soybean meal (47.5%CP)	28.5	28.5	28.5	28.5	28.5	28.5
Cornstarch	3.38	2.84	2.30	1.77	1.23	0.69
Soybean oil	2.0	2.0	2.0	2.0	2.0	2.0
Salt	0.33	0.33	0.33	0.33	0.33	0.33
Limestone	1.19	1.35	1.51	1.66	1.82	1.98
Monocalcium phosphate	0.0	0.38	0.76	1.14	1.52	1.90
Vitamin premix ¹	0.15	0.15	0.15	0.15	0.15	0.15
Mineral premix ²	0.1	0.1	0.1	0.1	0.1	0.1
Selenium premix ³	0.05	0.05	0.05	0.05	0.05	0.05
L-Lys HCl	0.15	0.15	0.15	0.15	0.15	0.15
DL-Met	0.10	0.10	0.10	0.10	0.10	0.10
L-Thr	0.05	0.05	0.05	0.05	0.05	0.05
Total	100.0	100.0	100.0	100.0	100.0	100.0
Calculated nutrients and energy						
CP, %	19.1	19.1	19.1	19.1	19.1	19.1
ME, kcal/kg	3420	3398	3377	3356	3334	3313
Total Ca, %	0.56	0.68	0.81	0.93	1.06	1.18
Total P, %	0.37	0.45	0.53	0.61	0.70	0.78
Apparent digestible P, g/kg	1.22	1.90	2.57	3.25	3.93	4.60
Standardized digestible P, g/kg	1.54	2.26	2.98	3.70	4.43	5.15
Ca:total P	1.52	1.52	1.52	1.52	1.52	1.52
Ca:apparent digestible P	4.58	3.60	3.14	2.86	2.69	2.56
Ca:standardized digestible P	3.64	3.03	2.71	2.51	2.38	2.29
Standardized ileal digestible AA, %						
Arg	1.13	1.13	1.13	1.13	1.13	1.13
His	0.46	0.46	0.46	0.46	0.46	0.46
Ile	0.69	0.69	0.69	0.69	0.69	0.69
Leu	1.44	1.44	1.44	1.44	1.44	1.44
Lys	0.99	0.99	0.99	0.99	0.99	0.99
Met	0.36	0.36	0.36	0.36	0.36	0.36
Met + Cys	0.63	0.63	0.63	0.63	0.63	0.63
Phe	0.81	0.81	0.81	0.81	0.81	0.81
Phe + Tyr	1.34	1.34	1.34	1.34	1.34	1.34
Thr	0.64	0.64	0.64	0.64	0.64	0.64
Trp	0.20	0.20	0.20	0.20	0.20	0.20
Val	0.75	0.75	0.75	0.75	0.75	0.75

¹Vitamin premix supplied per kilogram of diet: 3,630 IU vitamin A, 363 IU vitamin D₃, 36.4 IU vitamin E, 1.3 mg menadione, 23.1 µg vitamin B₁₂, 5.28 mg riboflavin, 13.1 mg D-pantothenic acid, and 19.8 mg niacin (Purdue University, West Lafayette, IN).

²Mineral premix supplied per kilogram of diet: 11.3 mg Cu (as copper chloride), 0.46 mg I (as ethylenediamine dihydroiodide), 121 mg Fe (as iron carbonate), 15 mg Mn (as manganese oxide), and 121 mg Zn (as zinc oxide; Purdue University).

³Supplied 300 µg of Se per kilogram of diet (Purdue University).

Table 3. Analyzed DM, N, Ca, and P composition of ingredients and diets (%)¹

Item	Ingredient				Diet, g standardized total-tract digestible P/kg					
	Corn	Soybean meal	Monocalcium phosphate	Limestone	1.54	2.26	2.98	3.70	4.43	5.15
DM	88.73	90.69	97.41	98.68	89.55	89.71	89.81	89.84	89.85	89.87
SD	0.53	1.85			0.59	0.51	0.55	0.49	0.45	0.51
N	1.28	7.34	0.13	0.10	3.06	3.04	2.98	2.99	3.06	3.00
SD	0.10	0.35			0.06	0.06	0.09	0.12	0.17	0.08
Ca	0.03	0.35	15.32	38.98	0.69	0.80	0.86	1.01	1.16	1.29
SD	0.004	0.07			0.08	0.13	0.15	0.10	0.15	0.18
P	0.30	0.72	21.10	0.07	0.41	0.49	0.58	0.65	0.77	0.85
SD	0.03	0.04			0.03	0.03	0.04	0.03	0.04	0.06

¹Values represent mean of samples sent from each of 10 universities.

Femurs and feet were allowed to thaw overnight and then scanned by DXA (small animal scan mode, software version 12.20, GE Lunar Prodigy; GE Healthcare, Waukesha, WI). Analysis of the scans was used to determine BMC (g ash/bone or foot) and BMD (g ash/cm²) as described earlier (Rortvedt and Crenshaw, 2012). Femurs were cleaned of adhering tissue and subjected to a 4-point bending test as described earlier (Aiyangar et al., 2010; Rortvedt and Crenshaw, 2012). Loads were applied at a constant deformation rate (5 mm/min) using an Instron Universal Testing Machine (model 5566; Instron Corp., Canton, MA) with a 1,000-kg load cell. During the mechanical test, the femur was supported in an anterior–posterior anatomical orientation during the load application and values from the recorded load-deformation curve were used to derive the maximum load at failure. All feet were shipped to the University of Kentucky for mechanical testing of the metacarpal bones.

Both left and right feet were thawed and the third and fourth metacarpals of each foot were removed. After dissection, the bones were frozen, later thawed, cleaned of extraneous soft tissue, and refrozen. Before breaking, the metacarpals were allowed to thaw for 5 to 6 h and then subjected to breaking strength determinations with an Instron Universal Testing Machine (Instron Corp.). Maximum load is defined as the peak amount of force, before fracture, applied by a wedge mounted on a pressure-sensitive compression cell at the center of the fresh bone when placed horizontally on two supports spaced 3.2 cm apart. Force was applied to the shaft of the bone at 5 cm/min and measured by a pressure sensitive cell and recorded on a graph recorder. The average of both left and right feet was used in the data analyses.

Nutrient Balance

Diets fed at 2 of the 10 participating stations were evaluated with 48 barrows (not a subset of pigs used in the growth performance study) in nutrient balance experiments with 8 individually fed barrows per diet to determine digestibility and retention of N, Ca, and P. Barrows

were blocked on the basis of initial BW and randomly assigned to the diets within each block at each station. The barrows were allowed 7 d to adapt to individual stainless-steel metabolism cages that allowed separate collection of feces and urine and to the feeding regime. This was followed by a 7-d period of total collection and separate collection of feces and acidified urine using protocols described by Adeola (2001). During the nutrient balance study, feed was provided at approximately 4% of BW during the 7-d collection period. The first and the last meal of the collection period had ferric oxide added to mark the initiation and end of fecal collection.

Chemical Analyses

Fecal samples were thawed and mixed thoroughly and subsamples were dried at 55°C for 72 h. Fecal subsamples and diets were air equilibrated and ground through a 1-mm screen. Samples of urine were thawed and strained through glass wool to remove particulate matter. Analyses of the diets, feces, urine, limestone, and MCP for DM (method 934.01; AOAC, 2006), N (method 976.05; AOAC, 2006), Ca (atomic absorption spectrometry; method 968.08; AOAC, 2006), and P (spectrophotometry at 620 nm; method 985.01(A, B, D); AOAC, 2006) were all conducted at 1 location (USDA-ARS, Ames, IA).

After maximum load strength measurement, both left and right metacarpals were cut in half to remove the marrow and dried in an oven. The dried bones were wrapped in cheesecloth and extracted with fresh petroleum ether three times at 24-h intervals. They were then air dried at room temperature under a chemical hood for 24 h, dried in an oven overnight, and then ashed in a muffle furnace at 600°C for at least 16 h (method 942.05; AOAC, 2003). Ash weight was recorded and the ash percent in dry, fat-free bone was determined. Following wet ashing, P was assessed by gravimetric procedures (modification of method 966.01; AOAC, 2003). Calcium concentration was assessed by flame atomic absorption spectrophotom-

Table 4. Growth performance of barrows and gilts fed diets containing 6 concentrations of standardized total-tract digestible (STTD) P

Item	Diet, g STTD P/kg						Sex					
	1.54	2.26	2.98	3.70	4.43	5.15	SEM	<i>P</i> -value	Barrow	Gilt	SEM	<i>P</i> -value
Number of replicates	40	40	40	40	40	40			120	120		
BW, kg												
d 0	19.0	18.9	19.0	18.8	18.9	19.1	–	–	19.1	18.8	–	–
d 14 ¹	26.9	27.6	28.1	28.6	29.2	29.2	0.27	<0.001	28.5	28.2	0.16	0.094
d 28 ^{1,2}	36.9	38.5	40.0	41.1	41.6	41.7	0.34	<0.001	40.3	39.6	0.20	0.006
ADG, g												
d 0 to 14 ^{1,2}	568	620	656	699	733	727	10.3	<0.001	673	662	5.92	0.177
d 14 to 28 ^{1,2}	710	779	844	887	886	891	11.4	<0.001	847	819	6.60	0.003
d 0 to 28 ^{1,2}	639	700	751	793	810	809	8.40	<0.001	760	740	4.85	0.004
ADFI, kg												
d 0 to 14 ^{1,2}	1.18	1.23	1.24	1.28	1.29	1.28	0.014	<0.001	1.25	1.25	0.008	0.619
d 14 to 28 ^{1,2}	1.59	1.65	1.71	1.75	1.75	1.76	0.024	<0.001	1.74	1.66	0.014	<0.001
d 0 to 28 ^{1,2}	1.39	1.45	1.48	1.52	1.52	1.52	0.017	<0.001	1.50	1.46	0.010	0.004
G:F, g/kg												
d 0 to 14 ^{1,2}	484	506	532	546	569	568	5.1	<0.001	537	531	2.947	0.170
d 14 to 28 ¹	464	477	497	503	507	505	9.5	0.004	487	497	5.462	0.187
d 0 to 28 ^{1,2}	470	490	513	522	535	535	4.8	<0.001	510	512	2.775	0.638

¹Linear effect of STTD P ($P < 0.01$).

²Quadratic effect of STTD P ($P < 0.05$).

etry at a wavelength of 422.7 nm (Thermo Elemental, SOLAAR M5; Thermo Electron Corp., Verona, WI) according to a modification of the AOAC (2003) procedure (method 927.02) using a Ca reference solution (1,000 mg/L; Fisher Scientific, Fair Lawn, NJ) for development of the standard curves. Similar to bone maximum load, the average of both left and right feet was used in data analyses.

Statistical Analyses

Participating stations sent growth performance, bone, and nutrient balance data to the study coordinator for statistical analyses using SAS (SAS Inst. Inc., Cary, NC). Pen was considered the experimental unit for the growth performance data and pig was considered the experimental unit for the bone and nutrient balance data. The initial statistical evaluation revealed a lack of interaction between diet and station. Subsequent analyses included station, diet, sex, and the interaction of diet and sex as possible sources of variation. As no interaction between diet and sex was detected, the interaction was pooled into the error term. Diet effect (increasing levels of STTD P) was further partitioned into linear and quadratic effects using orthogonal polynomial contrasts. An α level of 0.05 was considered statistically significant. Broken-line regression analyses were conducted to determine estimates of STTD P requirements using the NLIN procedure of SAS as described by Robbins et al. (2006).

RESULTS

Monocalcium phosphate and limestone were from the same batch but locally sourced corn and soybean meal were used to mix experimental diets at each participating station. Therefore, the DM, N, Ca, and P analyses of corn, soybean meal, and diets conducted at the same laboratory and presented in Table 3 represent an average of 10 participating stations. Analyzed concentrations of N, Ca, and P were within expectation and the average N concentrations of experimental diets were similar to the formulated 19% CP and consistent across diets (Table 3). The interaction between diet and sex was not significant; therefore, the main effects of diet and sex on growth performance are presented (Table 4). Pigs were allotted to the study at an average initial BW of 19 kg and there was a linear increase ($P < 0.05$) in BW as STTD P increased at d 14 or 28 of the study. Fourteen days into the study, barrows tended ($P < 0.1$) to be heavier than gilts, and at the end of the 28-d study, barrows were heavier ($P < 0.01$) than gilts. There were both linear ($P < 0.01$) and quadratic ($P < 0.05$) effects of STTD P on ADG from d 0 to 14, 14 to 28, and 0 to 28. During the 28-d growth from 19 to 40 kg BW, barrows gained faster and consumed more feed ($P < 0.01$) than gilts, but there was no difference in G:F, between barrows and gilts (Table 4). Increasing dietary concentrations of STTD P linearly ($P < 0.01$) and quadratically ($P < 0.05$) increased ADFI during d 0 to 14, 14 to 28, and 0 to 28. Furthermore, barrows had greater ($P < 0.01$) ADFI than gilts during d 14

Table 5. Bone mineralization of barrows and gilts fed diets containing 6 concentrations of standardized total-tract digestible (STTD) P

Item	Diet, g STTD P/kg						SEM	P-value	Sex			
	1.54	2.26	2.98	3.70	4.43	5.15			Barrow	Gilt	SEM	P-value
Number of replicates	12	12	12	12	12	12			36	36		
Final BW, kg ^{1,2}	37.6	40.0	41.8	43.4	43.9	43.6	0.72	<0.001	42.1	41.3	0.41	0.174
Femur												
Mineral density, g/cm ^{2 1,2}	0.385	0.464	0.561	0.672	0.717	0.761	0.0185	<0.001	0.585	0.602	0.0108	0.260
Mineral content, g/bone ^{1,2}	18.36	22.23	26.56	31.42	34.10	35.16	0.796	<0.001	27.59	28.35	0.465	0.249
Maximum load, N ¹	790	1,261	1,649	2,104	2,588	2,533	108.5	<0.001	1,815	1,827	63.4	0.902
Metacarpal												
Mineral density, g/cm ^{2 1}	0.338	0.345	0.381	0.430	0.445	0.454	0.0112	<0.001	0.399	0.398	0.0065	0.895
Mineral content, g/bone ¹	14.44	14.93	16.80	19.24	20.42	20.25	0.589	<0.001	17.82	17.54	0.340	0.562
Maximum load, N ¹	36.8	43.2	52.9	61.5	73	79.7	2.46	<0.001	57.6	58.2	1.42	0.780
Ash, % of dry, fat-free bone ^{1,2}	45.57	46.61	50.10	52.12	51.85	52.61	0.559	<0.001	49.78	49.84	0.322	0.895
Ca, % of bone ^{1,2}	17.58	17.89	19.05	19.83	19.67	19.91	0.198	<0.001	18.91	19.07	0.114	0.338
P, % of bone ^{1,2}	8.03	8.34	8.97	9.41	9.31	9.56	0.136	<0.001	8.88	8.99	0.079	0.023
Ca, % of bone ash ¹	38.58	38.39	38.01	38.04	37.95	37.85	0.105	<0.001	38.01	38.27	0.061	0.242
P, % of bone ash ¹	17.61	17.90	17.90	18.04	17.94	18.17	0.068	<0.001	17.83	18.02	0.039	0.002

¹Linear effect of STTD P ($P < 0.01$).

²Quadratic effect of STTD P ($P < 0.05$).

to 28 and 0 to 28. In the d 0 to 14 and 0 to 28 periods of the study, there were both linear ($P < 0.01$) and quadratic ($P < 0.05$) increases in G:F but only a linear increase ($P < 0.01$) in G:F from d 14 to 28 as concentrations of STTD P in the diets increased (Table 4).

Data on bone mineralization of pigs fed the 6 experimental diets containing increasing concentrations of STTD P are presented in Table 5. Final BW of the 12 pigs per diet (36 pigs per sex) that were selected for examination of the mineralization of the femur and the third and fourth metacarpal also linearly and quadratically increased ($P < 0.05$) with increasing dietary concentrations of STTD P. There were both linear and quadratic increases ($P < 0.05$) in mineral density and content of the femur as dietary STTD P increased (Table 5). Furthermore, the maximum load of the femur linearly increased ($P < 0.01$) with increasing dietary concentrations of STTD P. The data for metacarpals presented in Table 5 represent the average of the third and fourth metacarpals for both left and right feet. The mineral density and content and maximum load as well as the Ca and P expressed as a percentage of metacarpal ash linearly increased ($P < 0.01$) with increasing dietary concentrations of STTD P. There were both linear and quadratic increases ($P < 0.05$) in ash, Ca, and P expressed as a percentage of dry, fat-free metacarpal as dietary STTD P increased (Table 5).

Feed was provided at approximately 4% of BW during the 7-d collection period of the nutrient balance study and, thus, initial and final BW were not affected by STTD P of the diets (Table 6). Apparent digestibility and retention of N were not different across dietary treatments. However, apparent digestibility and reten-

tion of Ca linearly ($P < 0.01$) increased with increasing dietary concentrations of STTD P. For P, there were both linear and quadratic increases ($P < 0.01$) in apparent digestibility and retention with increasing concentrations of STTD P in the diets (Table 6). The calculated and determined STTD P and apparent total-tract digestible (ATTD) P are presented in Fig. 1. The calculated values were derived from ingredient information provided by the NRC (2012) and were used in formulating the diets. The determined values were based on data presented in Tables 3 and 6, together with a presumed basal fecal endogenous P loss of 0.19 g/kg DMI from the NRC (2012). At the lower end of STTD P concentration, determined values were numerically less, but at the higher end of STTD P concentration, determined values were numerically greater than calculated values. However, statistical analysis using the t-test procedure for a test of null hypothesis, H_0 : determined = calculated, revealed that there was no difference between the determined and calculated values presented in Fig. 1.

Breakpoints determined from nonlinear broken-line regression analyses of responses on STTD P concentrations to estimate requirements are presented in Fig. 2 and Table 7. Using ADG as response criterion, the STTD P requirement was estimated to be 4.20 ± 0.102 , 3.23 ± 0.036 , or 3.87 ± 0.090 g/kg for d 0 to 14, 14 to 28, or 0 to 28, respectively. Corresponding estimates using G:F as the response criterion were 4.34 ± 0.146 , 3.38 ± 0.139 , or 4.08 ± 0.195 g/kg (Fig. 2). When mineralization of the femur was used as criteria of response, estimates of STTD P requirement were 4.28, 4.28, or 4.34 g/kg for mineral density, mineral content, or maximum

Table 6. Apparent total-tract digestibility and retention of DM, N, Ca, and P in pigs fed diets containing 6 concentrations of standardized total-tract digestible (STTD) P

Item	Diet, g STTD P/kg						SEM	P-value
	1.54	2.26	2.98	3.70	4.43	5.15		
Number of replicates	8	8	8	8	8	8		
Initial weight, kg	25.4	25.6	25.5	25.1	24.8	24.7	0.41	0.284
Final weight, kg	34.3	34.3	34.2	33.3	33.7	33.5	0.46	0.488
DMI, g/7 d	6,938	6,964	6,972	6,962	7,029	7,027	70.1	0.917
N								
Intake, g/7 d	230.7	238.8	224.3	225.1	226.8	229.9	2.23	<0.001
Absorbed, g/7 d	194.3	202.9	189.3	189.0	191.5	189.5	2.72	<0.001
Retained, g/7 d	139.2	144.1	140.6	134.1	132.7	131.4	6.59	0.716
Apparent digestibility, %	84.3	85.0	84.4	84.0	84.3	82.4	1.23	0.749
Retention, %	60.5	60.1	62.4	59.6	57.8	56.6	2.89	0.770
Ca								
Intake, g/7 d ^{1,2}	61.6	69.2	73.3	90.0	103.0	113.6	1.30	<0.001
Absorbed, g/7 d ¹	26.5	33.4	41.1	48.5	62.8	69.3	2.95	<0.001
Retained, g/7 d ¹	19.6	27.2	36.2	43.2	57.5	63.8	2.93	<0.001
Apparent digestibility, % ¹	43.1	48.7	56.0	54.5	61.2	61.3	3.12	<0.001
Retention, % ¹	32.4	39.7	49.4	48.7	56.1	56.4	3.12	<0.001
P								
Intake, g/7 d ^{1,2}	29.5	36.5	42.2	48.7	57.3	66.4	0.57	<0.001
Absorbed, g/7 d ¹	5.9	11.1	19.7	23.8	30.4	35.9	1.62	<0.001
Retained, g/7 d ¹	5.8	10.9	19.5	23.6	30.3	35.8	1.63	<0.001
Apparent digestibility, % ^{1,2}	20.3	30.5	46.6	49.2	53.2	54.3	2.96	<0.001
Retention, % ^{1,2}	19.8	30.2	46.2	48.8	53.0	54.0	2.96	<0.001

¹Linear effect of dietary P ($P < 0.01$).

²Quadratic effect of dietary P ($P < 0.01$).

load, respectively (Table 7). Using the mineralization of metacarpal response criteria listed in Table 7, estimates of STTD P requirement ranged from 3.50 to 5.01 g/kg with a 95% confidence interval of 2.33 to 5.59 g/kg. Because of the notably small urine excretion of P, estimates of STTD P requirements were the same for both absorbed and retained P at 4.99 g STTD P/kg diet with a 95% confidence interval of 4.43 to 5.54 g/kg (Table 7).

DISCUSSION

The analyzed DM, N, Ca, and P of corn and soybean meal used at the 10 participating stations showed that the CV in the DM was less than 1% for corn and approximately 2% for soybean meal. Coefficients of variation for N in corn or soybean meal across the 10 stations were approximately 8 or 5%, respectively. The CV of Ca was 13 and 20% and 10 and 6% for P in the corn and soybean meal, respectively, used at all participating stations. In diets, analyzed concentrations of N, Ca, and P were within expectation and the average N concentration of experimental diets were similar to the formulated 3.04% N and consistent across diets. Initial statistical evaluation of the growth performance data indicated an absence of interactions among station and diet and diet and sex; hence, the main effects of diet and sex were

Table 7. Estimates of standardized total-tract digestible (STTD) P requirements, g/kg

Response variable	Estimate of STTD P requirement, g/kg	SE	95% confidence limits
Femur			
Mineral density	4.28	0.195	3.89 to 4.67
Mineral content	4.28	0.211	3.86 to 4.70
Maximum load	4.34	0.226	3.89 to 4.79
Metacarpal			
Mineral density	4.52	0.384	3.76 to 5.29
Mineral content	4.34	0.442	3.46 to 5.22
Maximum load	5.01	0.289	4.43 to 5.59
Bone ash	3.75	0.218	3.32 to 4.19
Bone Ca	3.72	0.243	3.23 to 4.20
Ca of bone ash	3.89	0.703	2.49 to 5.29
Bone P	3.75	0.211	3.33 to 4.17
P of bone ash	3.50	0.583	2.33 to 4.67
P utilization			
Absorbed	4.99	0.276	4.43 to 5.54
Retained	4.99	0.276	4.43 to 5.54

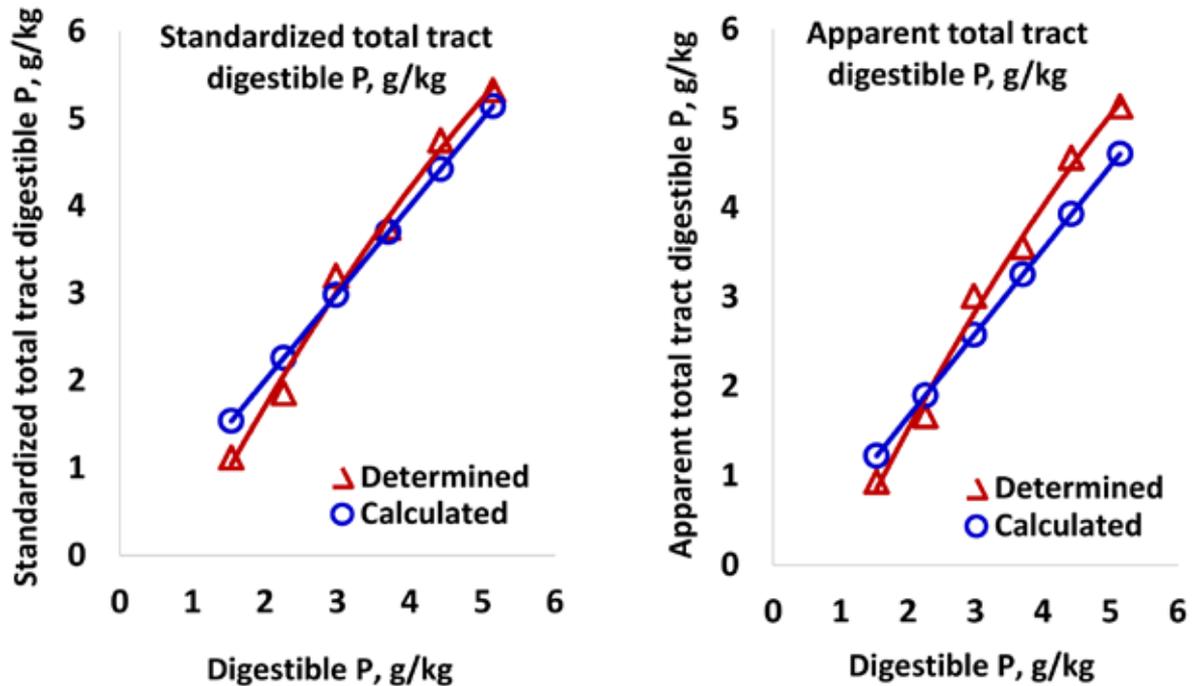


Figure 1. Relationship between determined and calculated standardized (left panel) or apparent (right panel) total-tract digestible P for 6 dietary concentrations of digestible P fed to pigs.

presented and will now be discussed. Phosphorus has more known functions than any other mineral element in the body and primary functions include roles in mineralization of connective tissues, components of nucleic acids, and nucleotide phosphates as well as phospholipids and active sites of enzyme and transport proteins among others (Crenshaw, 2001). Predictably, increasing dietary concentrations of STTD P in diets spanning a P range from 47 to 156% of the NRC (2012) requirement estimate linearly increased weight gain, feed intake, and feed efficiency during the d 0 to 14, 14 to 28, and 0 to 28 periods of the current study. This is consistent with the copious roles of P in the body for which improved growth performance follows alleviation of P deficiency. In earlier studies, weight gain and feed efficiency increases were reported in pigs that received diets containing increasing concentrations of P (Ketaren et al., 1993; Eeckhout et al., 1995; Sands et al., 2001; Ekpe et al., 2002). Observations in the current study are consistent with the results of recent studies reported by Almeida and Stein (2010), Partanen et al. (2010), Saraiva et al. (2012), and Zhai and Adeola (2013a,b) that showed improved growth performance of pigs when offered diets formulated to contain graded levels of STTD P.

Within the body, 60 to 80% of P is contained in the skeletal tissue and, along with Ca, is continuously deposited and reabsorbed from bone throughout life, thus playing key roles in mineralization of bones (Crenshaw, 2001). This prompted the decision to evaluate mineralization responses of femur and metacarpal to graded

intake of STTD P in the current experiments. Femur mineralization responded to increased STTD P intake with increases in mineral density and content as well as increased maximum load. In studies conducted to determine the quantitative relationship between femur mineral content and STTD P intake in 22-kg pigs, Gutierrez et al. (2015) reported increased femur mineral content with STTD P intake, which is consistent with the observed increase in femur mineral content response to increased STTD P intake in the current study. A regression of the maximum load on femur mineral content (data in Table 6) for the 72 femur samples indicated that the regression equation generated (maximum load = $-116 + 10.8 \times \text{mineral content}$) explained 98% of the variation in the data set, demonstrating that DXA-generated mineral content is highly correlated with maximum load. These results confirm earlier observations (Aiyangar et al., 2010) that detected positive correlations between bone strength and whole-body BMC of growing pigs fed diets deficient in Ca and P or diets that allowed a recovery from the deficiency. Lower correlations were observed between whole-body BMD and femur strength.

Consistent with the observations reported by Maxson and Mahan (1983), O'Doherty et al. (2010), and Petersen et al. (2011), increased intake of digestible P was accompanied, in the current study, by increases in mineralization of metacarpals. Mineral density and content, maximum load, and ash of metacarpals increased in pigs as STTD P increased from 1.54 to 5.15 g/kg of diet. Presumably, increased intake of STTD P, together with

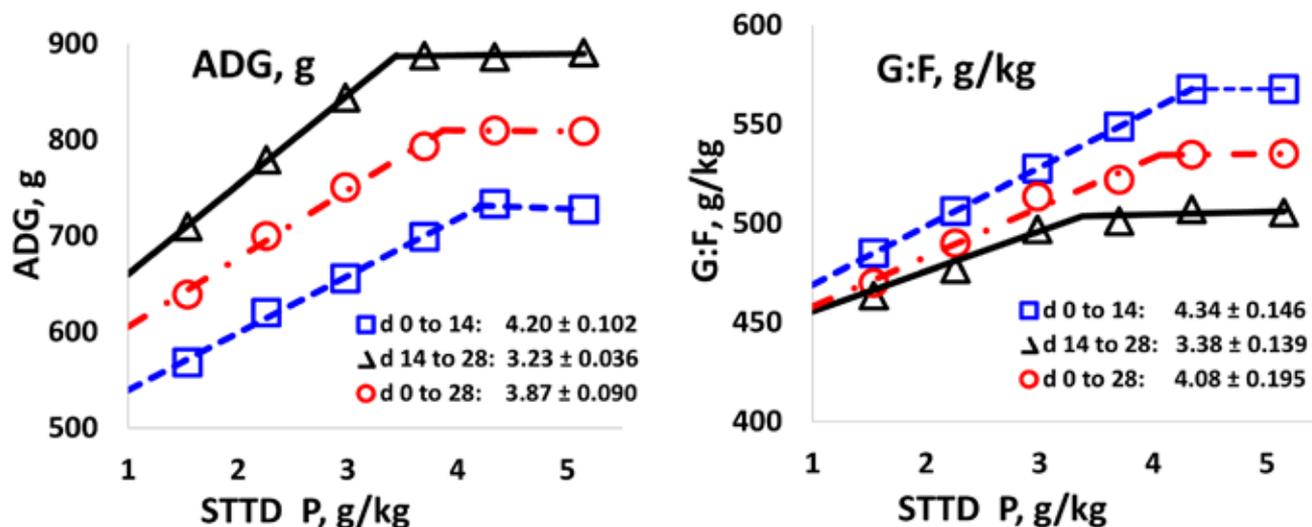


Figure 2. Standardized total-tract digestible (STTD) P requirement (g/kg \pm SE) of pigs using broken-line regression analysis for ADG or G:F data during d 0 to 14, 14 to 28, or 0 to 28 on an as-fed basis. Each data point represents a mean of 40 replicate pens of barrows and gilts.

Ca, was used for synthesis and deposition of hydroxyapatite in the metacarpals and hence the increase in bone mineralization. As in the femur, the equation generated (maximum load = $-5.1 + 0.623 \times$ mineral content) from the regression of maximum load on metacarpal mineral content for the 72 metacarpals explained 94% of the variation in the data set, again demonstrating that in this bone, DXA-generated mineral content is highly correlated with maximum load. Furthermore, a regression of metacarpal ash on metacarpal mineral content for the 72 metacarpals indicated that the regression equation generated (metacarpal ash = $30.5 + 1.09 \times$ mineral content) explained 93% of the variation in the data set, indicating that DXA-generated mineral content is highly correlated with metacarpal ash. The bone ash is expressed on a dry fat-free basis as a means of reducing the variation that accompanies changes in fat and water contents associated with nutritional status and age. Using an equation derived from the regression of metacarpal ash on metacarpal mineral content to predict femur ash produced 50.6, 54.8, 59.5, 64.8, 67.8, and 68.9% femur ash, which, at the higher STTD P intake, are similar to the reported 64 to 69% dry, fat-free ash contents of the femur (Vose and Kubala, 1959; Sauer et al., 2009) and the 68 to 71% dry, fat-free ash contents of the humerus (Crenshaw et al., 2013). The observation that metacarpal ash contained 17 to 18% P and 38 to 39% Ca in the current experiment is consistent with previous reports for dry fat-free bone ash and the notion that hydroxyapatite of the bone contains Ca and P in a ratio of approximately 2.2:1.0 (Crenshaw, 2001; Baker et al., 2013; Crenshaw et al., 2013). In the current study, Ca:P ratios in metacarpal ash were 2.13:1.0 or 2.12:1.0 for barrows or gilts, respectively, and this ratio decreased from 2.19:1.0 to 2.08:1.0 in metacarpals of pigs as STTD P increased from 1.54 to 5.15 g/kg of diet, indicating hy-

droxyapatite sensitivity, dynamic nature, and ion substitution patterns during mineral accumulation.

Corn and soybean meal supplied all the organic P in the 6 diets and graded levels of MCP were added to attain desired dietary P concentrations. Furthermore, limestone was added to increase dietary Ca concentrations and maintain Ca:P ratio at 1.52:1.0. Phosphorus balance studies conducted with diets at 2 stations indicated that calculated ATTD P as well as STTD P was not different from determined values. Digestibility and retention of Ca or P increased linearly or both linearly and quadratically, respectively, to graded levels of limestone and MCP at the fixed Ca:P ratio of 1.52:1.0, which are consistent with previous reports (Partanen et al., 2010; Petersen et al., 2011). In recent studies, increased ATTD P was reported when pigs were offered diets containing graded additions of MCP (Zhai and Adeola, 2013a,b; Gutierrez et al., 2015). The improved digestibility of P in diets with the progressive supplementation of MCP is due to greater availability of P in MCP compared with that in corn and soybean meal (Zhai and Adeola, 2013a). The apparent total-tract digestibility of P in the corn-soybean meal diet in the current study is similar to that reported in Zhai and Adeola (2013b). Regressing P absorbed on P intake generated the following equation: $y = 0.8277x - 17.576$ ($R^2 = 0.9808$), indicating that the digestibility of P in MCP used in the current study is 83%, which is similar to the 84% digestibility of P in MCP reported by Zhai and Adeola (2013b). A similar regression of absorbed P on P intake of the data published by Gutierrez et al. (2015) also generated the equation $y = 0.8262x - 2.2295$ ($R^2 = 0.9864$) and 83% P digestibility in MCP. These digestibility values of P in MCP from the different experiments closely compare with the 83% digestibility of P in MCP published by the NRC (2012).

In the current study, urine excretion of P was notably small, being less than 30 mg/d across the 6 dietary concentrations of STTD P from 1.54 to 5.15 g/kg of diet, and therefore, the retained P response of pigs to dietary STTD P was similar to the absorbed P response.

Estimates of STTD P requirements of 20- to 40-kg pigs were determined using growth performance, bone mineralization, and P balance as criteria of response in the current experiments. Routine empirical experiments that provide estimates of requirements reflect the changes over time in swine genetics, feedstuffs, and environment, which allow updates of databases and are valuable for the validation of the estimates generated from the factorial approach. That STTD P requirements for P retention and bone mineralization were generally greater than those for growth performance in the current experiments confirm previous reported observations (Cromwell et al., 1970; Ketaren et al., 1993; Partanen et al., 2010). These are consistent with the notion that deposition of minerals in bones continues past the attainment of maximum muscle gain (Crenshaw, 2001). The requirement for STTD P to support P retention was 4.99 g/kg and that to support femur and metacarpal mineralization was 4.28 to 4.34 g/kg. The data reported by Gutierrez et al. (2015) had a breakpoint for femur mineralization at 8.84 g STTD P/d, which at 1.6 kg feed/d translates to 5.5 g STTD P/kg diet. Using ADG and G:F as response criteria, the mean STTD P requirement was estimated to be 4.27 g/kg (mean of 4.2 and 4.34) during the d 0 to 14 period of the study (19 to 28 kg BW), 3.31 g/kg during the d 14 to 28 period of the study (28 to 40 kg BW), and 3.98 g/kg during the d 0 to 28 period of the study (19 to 40 kg BW). When published data are recalculated, Jongbloed and Everts (1992) observed digestible P requirements of 3.2 and 2.3 g/kg for 25- and 30-kg pigs, respectively; Ekpe et al. (2002) observed digestible P requirement of 3.4 g/kg for pigs raised from 24 to 61 kg BW; for 21- to 39-kg pigs, Ruan et al. (2007) observed digestible P requirement of 3.4 g/kg; and Zhai and Adeola (2013b) observed digestible P requirement of 3.4 g/kg for pigs raised from 20 to 32 kg BW. Furthermore, the estimates generated from NRC (2012) models are 3.3, 3.1, and 3.1 g/kg for 20 to 28, 28 to 40, and 20 to 40 kg BW, respectively. Perhaps several factors are responsible for the discrepancy in estimates of STTD P requirements for growth performance between the current study and those stated above, the central one being the total dietary Ca concentration for the total Ca:STTD P ratio at which there was a breakpoint (requirement estimate) for STTD P. For the current study and those cited above, the breakpoint occurred at a total Ca:STTD P ratio of approximately 2.50:1.0. However, the total dietary Ca concentration at which the breakpoint occurred was at least 10 g/kg in the current study, whereas total dietary

Ca concentrations at which the breakpoint occurred in the reports cited above were between 6.5 and 8.5 g/kg. Therefore, total dietary Ca was likely a limiting nutrient in the reports cited above, but not in the current study.

From the foregoing, the current cooperative study provided empirical estimates of STTD P requirements of 20- to 40-kg pigs. These estimates from nonlinear regression using broken-line analyses were 4.20 ± 0.102 , 3.20 ± 0.036 , or 3.87 ± 0.090 g/kg for ADG during d 0 to 14, 14 to 28, or 0 to 28, respectively, and 4.34 ± 0.146 , 3.38 ± 0.139 , or 4.08 ± 0.195 g/kg for G:F during d 0 to 14, 14 to 28, or 0 to 28, respectively.

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