Effects of feeding corn-expressed phytase on the live performance, bone characteristics, and phosphorus digestibility of nursery pigs

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ABSTRACT: A 41-d feeding trial was conducted to determine the efficacy of a corn-expressed phytase (GZ; GraINzyme, Agrivida Inc., Woburn, MA) on the live performance, bone characteristics, and P digestibility of nursery pigs fed a reduced P diet. Weaned piglets (21 ± 3 d; *n* = 324) were acclimated on a common diet for 7 d (phase 1) before randomization into 54 single-sex pens (5 gilt and 4 barrow pens per treatment) containing 6 pigs (6.6 ± 1.2 kg) per pen. Six treatments were fed: positive control (**PC**; 0.4% or 0.32% aP for phase 2 or 3 and 4, respectively), negative control (**NC**; 0.15% reduction in aP), and 500, 1,000, 2,000, or 4,000 FTU per kg phytase from GZ added to NC in a 3-phase feeding program. Pigs were weighed on day 1, 14, 28, and 41, and feed disappearance recorded per phase. Apparent total tract digestibility (ATTD) of P was determined by feeding chromic oxide marker (day 28 to 35) and collecting fecal samples on day 35. On day 41, 4 pigs per pen were euthanized and metacarpal bones were collected to evaluate bone breaking strength (**BBS**) and ash. Data were analyzed using PROC GLM of SAS (block, sex, and treatment). Treatment least squares means were separated and linear and quadratic treatment effects evaluated. Other than feed efficiency (G:F) and day 15 to 28 ADFI, the pigs fed PC were superior ($P < 0.05$) to NC-fed pigs in all other variables. Pigs fed ≥500 FTU per kg phytase had increased ($P < 0.05$) ADG and ADFI compared to NC pigs and equivalent ($P > 0.05$) ADG and ADFI as PC pigs from day 0 to 41. Feeding ≥500 FTU per kg phytase resulted in higher ($P < 0.05$) ATTD of P than both NC and PC pigs and higher ($P < 0.05$) BBS and bone ash weight than NC. Pigs fed 1,000 or 2,000 FTU per kg phytase had equivalent ($P > 0.05$) BBS and bone ash weight compared to pigs fed PC diets. Feeding 4,000 FTU per kg phytase resulted in higher ($P < 0.05$) day 1 to 41 ADG, ATTD of P, and bone ash weight compared to feeding ≤1,000 FTU per kg phytase or PC diets. There were linear ($P < 0.05$) increases in ADG, ADFI, ATTD of P, BBS, and bone ash characteristics as GZ inclusion increased. In conclusion, ≥500 FTU per kg phytase from GZ improved growth, ATTD of P, BBS, and bone ash when added to a reduced P diet and 4,000 FTU per kg phytase increased growth greater than the PC treatment.

Key words: bone parameters, corn-expressed phytase, nursery pig, performance, phosphorus digestibility

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INTRODUCTION

Supplementing pig diets with phytase has been shown to make the phytate-bound P available for absorption, as demonstrated by increased P digestibility (Bento et al., 2012; Rutherfurd et al., 2014). Increased P availability to the animal has also been shown with the improvement in bone mineralization when phytase is added to reduced P diets (Braña et al., 2006; Veum et al., 2006; Torrallardona and Ader, 2016). The response of P (and Ca) reduction, and subsequent phytase

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addition, on animal performance has been variable. For example, sometimes there is a G:F but not ADG response (Braña et al., 2006) or neither response is significantly different (Torrallardona and Ader, 2016) or there is a performance response but not a percent bone ash difference (Santos et al., 2014).

A corn-expressed phytase (660 FTU per g) was developed and tested previously in pigs by Nyannor et al. (2007), displaying linear increased ADG and G:F with phytase addition and an equivalent response to a microbial produced phytase. Corn expressing a lower concentration of phytase (7.134 FTU per g; Li et al., 2013) has also been tested, completely replacing all of the corn in the diet, resulting in increased energy and P digestibility when compared to conventional corn.

The corn-expressed phytase (GraINzyme, Agrivida Inc., Woburn, MA) tested in this current research has been shown to be safe and effective in poultry (Ligon, 2016) and contains an engineered *Escherichia coli* phytase called Phy02. GraINzyme (**GZ**) has been demonstrated to improve ADG, feed efficiency, bone mineralization and strength, and P digestibility when young pigs were fed reduced P and Ca diets (Lee et al., 2017; Blavi et al., 2018; Knapp et al., 2018a, 2018b; Munoz Alfonso et al., 2018). The objective of this study was to determine the efficacy of GZ phytase on the live performance, bone characteristics, and P digestibility of nursery pigs fed a reduced P and adequate Ca diet. Reducing only dietary P will increase Ca to P ratio; therefore, we hypothesize the phytase response will remain linear up to the highest phytase level fed.

MATERIALS AND METHODS

All aspects of the study were conducted in accordance with the *Guide for Care and Use of Agricultural Animals in Research and Teaching* (FASS, 2010).

Animals and Housing

At weaning (21 ± 3 d), a group of 360 Danbred pigs (barrows and gilts) were sourced from a commercial swine operation (Prairie View Pork, Drexel, MO). Pigs were received and placed on a common medicated commercial phase I diet (Lean Start 2 Complete, Hubbard Feeds, Mankato, MN) for 7 d. Pigs were housed in a tunnel-ventilated commercial research nursery containing 64 pens with plastic grate floors. Each pen was 1.65 m × 1.65 m and contained one adjustable-height spiglett water lixit, one 38 cm Smidley stainless steel feeder, and contained one 20 cm gruel pan for 3 d after arrival. The building contained radiant tube heaters, exhaust fans, vent boards, misters, and a curtain controlled air inlet.

Due to greater ratio of gilts than barrows received, 1 pen of barrows within each treatment was removed from the study design. At the end of the acclimation phase, pigs were weighed (6.6 ± 1.2 kg) and randomized into 54 single sex pens (5 gilt and 4 barrow pens per treatment) with each pen containing 6 pigs.

Treatments

The study consisted of 6 treatment diets: positive control (**PC**) with adequate P (NRC, 2012), negative control (**NC**) with non-phytate P reduced by 0.15%, and NC plus either 500, 1,000, 2,000, or 4,000 FTU phytase per kg supplied by GZ. The pigs were fed for two 14-d phases (phase 2 and 3) and one 13-d phase (phase 4). One basal diet (PC) was formulated and mixed, for each dietary phase (Table 1), without the inclusion of monocalcium phosphate. Complete treatment diets were then mixed with monocalcium phosphate added for the PC diet and limestone and corn added for the NC diets. The GZ was added to the phytase treatment diets at the expense of standard (#2) corn. Diets were formulated to meet the nutrient specifications of NRC (2012), other than P and Ca in NC diets, and mixed at University of Arkansas (Fayetteville, AR). Feed placements to pens were done on an "as needed" basis and the pigs were provided ad libitum access to feed and water.

Sample Collection and Analysis

Basal diet samples (1 per feeding period) were sent to a commercial laboratory (Eurofins Scientific, Des Moines, IA) for moisture, crude protein, crude fat, lysine and crude fiber (methods 930.15, 990.03, 920.39, 975.44 and Ba 6a-05, respectively; AOAC, 2006). Individual diet samples from each period were also submitted to same lab for Ca and P (methods 965.17/985.01; AOAC, 2006) and phytase (method 2000.12; AOAC, 2006) analysis. The individual diet samples were also submitted to a different lab (Agrivida Inc., Woburn, MA) for phytase analysis, using an adjusted extraction procedure that employed an optimized buffer and extraction process (Li and Raab, 2016).

Individual pig body weight (BW) was measured at the beginning and at the end of each

Table 1. Composition of positive (PC) and negative (NC) control basal diet and calculated and analyzed nutrient values, as-fed basis

Growth phase:	Phase 2		Phase 3		Phase 4	
Diet:	PC	NC	PC	NC	PC	NC
Ingredients, %						
Corn	35.77	36.17	47.79	48.18	47.39	47.78
Soybean meal	30.000	30.000	31.600	31.600	31.600	31.600
Corn DDGS, 6% to 9% oil	15.000	15.000	15.000	15.000	15.000	15.000
Poultry fat	2.500	2.500	2.500	2.500	2.500	2.500
Monocalcium phosphate	0.725	0.000	0.700	0.000	0.700	0.000
Limestone	1.025	1.350	0.950	1.260	0.950	1.260
Salt	0.350	0.350	0.500	0.500	0.500	0.500
L-Lysine	0.365	0.365	0.345	0.345	0.340	0.340
DL-Methionine	0.153	0.153	0.106	0.106	0.105	0.105
L-Threonine	0.060	0.060	0.059	0.059	0.057	0.057
Trace mineral premix[1]	0.150	0.150	0.150	0.150	0.150	0.150
Vitamin premix[2]	0.250	0.250	0.250	0.250	0.250	0.250
Plasma	1.500	1.500	0.000	0.000	0.000	0.000
Milk, whey powder	12.000	12.000	0.000	0.000	0.000	0.000
Ethoxyquin	0.030	0.030	0.030	0.030	0.030	0.030
Tylan 40 Sulfa-G[3]	0.125	0.125	0.000	0.000	0.000	0.000
Tylan 40[4]	0.000	0.000	0.025	0.025	0.025	0.025
Chromic oxide	0.000	0.000	0.000	0.000	0.400	0.400
Calculated nutrient values						
ME, kcal/kg	3,413	3,426	3,400	3,413	3,387	3,400
CP, %	24.490	24.523	23.636	23.668	23.596	23.628
Total Lys, %	1.608	1.609	1.462	1.463	1.457	1.458
SID Lys, %	1.422	1.422	1.275	1.276	1.270	1.271
Total P, %	0.654	0.502	0.586	0.440	0.585	0.439
STTD P, %	0.403	0.250	0.322	0.174	0.321	0.174
Ca, %	0.756	0.756	0.654	0.653	0.654	0.653
Analyzed composition, %[5]						
DM	12.00		12.66		12.61	
CP	24.00		22.92		22.43	
Fat	5.65		6.20		6.76	
Lys	1.64		1.57		1.55	
Fiber	2.60		3.00		2.70	

[1]Supplied the following per kilogram of complete diet: 110 mg of Fe as ferrous sulfate; 110 mg of Zn as zinc sulfate; 33 mg of Mn as manganous oxide; 16.5 mg of Cu as copper sulfate; 0.30 mg of I as calcium iodate; 0.30 mg of Se as sodium selenite.

[2]Supplied the following per kilogram of complete diet: 8,820 IU of vitamin A; 2,205 of vitamin D_3; 44.1 IU of vitamin E; 38.6 μg of vitamin B_{12}; 4.4 mg of vitamin K as menadione nicotinamide bisulfite; 8.3 mg of riboflavin; 27.6 mg of pantothenic acid as D-calcium pantothenate; 82.7 mg of niacin.

[3]Tylan 40 Sulfa-G (Elanco Animal Health, Indianapolis, IN) provided at 110 mg tylosin and 110 mg sulfamethazine per kg of diet.

[4]Tylan 40 (Elanco Animal Health, Indianapolis, IN) provided 22 mg tylosin per kg of diet.

[5]Basal diet without monocalcium phosphate addition for PC diet or additional limestone and corn for NC diet.

dietary phase (day 14, 28, and 41) and ADG calculated for each growth phase and overall experimental feeding period. Pen feed consumption was measured daily and ADFI and G:F was calculated for each phase and the overall experimental feeding period.

Diets fed during the last phase contained chromic-oxide and fecal samples were collected from 4 random pigs per pen 7 d after the initiation of the phase (day 35) to determine apparent P digestibility. Fecal samples were collected by stimulating the anus to the pig until it defecated. The 4 samples per pen were placed into a plastic bag and mixed. The corner of the bag was cut with scissors, and 2 representative samples squeezed onto waxed paper. The wax paper was rolled and the sample placed into a 50 mL Falcon tube and frozen at −25 °C. The samples were then lyophilized in a vacuum-freeze drier (Genesis, Virtis, Gardiner, NY). The dried fecal (day 35) and phase 4 feed samples were assayed at a commercial lab (Whitbeck Labs, Springdale, AR) for P and chromium (methods 965/985.01; AOAC, 2006). Apparent P digestibility was calculated as described by Ajakaiye et al. (2003) using equation:

$$\text{Apparent P digestibility} = 100\% - [(Cr_d \times P_f)/(Cr_f \times P_d)] \times 100\%$$

where Cr_d is analyzed chromium in the diet (mg/kg), P_f is analyzed P in the feces (g/kg), Cr_f is analyzed chromium in the feces (mg/kg), and P_d is analyzed P in the diet (g/kg).

At the end of the live phase (day 41), 4 pigs per pen were randomly selected and humanely euthanized by captive-bolt gun. At the time of euthanasia, the right foot was collected, placed into a prelabeled zip-lock bag, and frozen at −25 °C until dissection. Upon dissection, the 4th metacarpal were placed into 50 mL Falcon tubes and returned to the freezer. Bones were analyzed (Louisiana State University, Baton Rouge, LA) for bone breaking strength (**BBS**), bone ash weight and percent bone ash (based off dry, defatted bone weight). Freshly thawed metacarpals were analyzed for BBS (HD 250 Texture Machine, Texture Technologies Corporation, Scarsdale, NY) using a 3-point bend rig with a load cell capacity of 250 kg and cross-head speed of 100 mm/min. After determining BBS, fat was extracted from metacarpals by a 48-h soxhlet extraction in ethyl alcohol followed by a 48-h extraction with diethyl ether. The bones were dried at 110 °C for 24 h and weighed. The dry, defatted bones were dry-ashed in a muffle furnace at 560 °C for 48 h and ash weight measured and the percent ash weight was calculated.

Statistical Analysis

The study followed a randomized complete block design (**RCBD**). Blocks were formed using initial pig weight (study day 1) and pen location within the building. Four blocks contained 12 pens, including 6 gilt and 6 barrow pens, and 1 block that contained 6 gilt pens. Within each sex and each block, 1 pen was assigned to each treatment diet. Pen served as the experimental unit and data were analyzed as a RCBD using the GLM Procedure of SAS (SAS Inst., Inc., Cary, NC). Least squares (LS) means were separated using the PDIFF option, and were considered significant if both the probability value of the effect in the model and the LS means differences were $P < 0.05$. In addition, orthogonal contrasts for phytase inclusion rate (linear and quadratic) were evaluated.

RESULTS

The analyzed nutrient profiles of the basal diets were similar to the formulated target levels as shown in Table 1. The analyzed Ca, P, and phytase levels for each treatment feed for all 3 phases are shown in Table 2. The analyzed calcium levels were variable across treatments; however, similar analytical variability has been reported previously (Gourley et al., 2018; ranging from 0.75% to 0.89% for a diet formulated for 0.73% Ca) when limestone or monocalcium phosphate were added to a basal diet. With the use of 1 basal diet mixed per phase, authors believe the variability is inherent within feed sample. Feed phytase levels were analyzed independently by 2 different laboratories, Agrivida Inc. (Medford, MA) and Eurofins (Des Moines, IA). While some of the phytase results vary between the 2 laboratories, they are within the variation as reported previously

Table 2. Analyzed calcium, phosphorus, and phytase in individual treatment diets (as-fed basis)

	Growth phase 2				Growth phase 3				Growth phase 4			
	Ca	P	Phytase		Ca	P	Phytase		Ca	P	Phytase	
Diet	%		FTU[1]	FTU[2]	%		FTU[1]	FTU[2]	%		FTU[1]	FTU[2]
PC[3]	0.779	0.70	<60	43	0.626	0.64	63	0	0.656	0.61	<60	69
NC[3]	0.703	0.55	<60	4	0.566	0.50	<60	0	0.604	0.48	<60	26
500[4]	0.702	0.54	304	212	0.557	0.49	705	376	0.566	0.49	235	658
1,000	0.736	0.54	902	390	0.634	0.48	961	989	0.530	0.47	354	814
2,000	0.629	0.55	1,850	1,636	0.571	0.47	1,240	2,222	0.603	0.46	2,170	1,712
4,000	0.679	0.55	4,710	2,792	0.560	0.49	2,590	3,529	0.594	0.48	3,330	3,006

[1]Analyzed by Eurofins, Des Moines, IA.

[2]Analyzed by Agrivida Inc., Medford, MA.

[3]PC = positive control; NC = negative control (−0.15% P).

[4]GraINzyme (Agrivida Inc., Woburn, MA) phytase units per kg of NC diet.

(Engelen et al., 2001) between different laboratories that independently tested the same samples.

The animal performance results for each period and overall are presented in Table 3. Sex affects were significant ($P < 0.05$) for ADG during phase 4 and overall, BW (day 1, 14, and 41), and bone ash weight, with barrows being greater than gilts. Treatment effects were significant ($P < 0.05$) for all variables measured except initial BW and G:F during phases 2 and 3 or overall (1 to 41 d).

After 14 d on treatment diets (phase 2), the NC pigs had lower BW than the other pigs ($P < 0.05$) and they remained lighter than the other pigs through the end of the experimental feeding period (day 41; $P < 0.05$). During each feeding phase, there was a significant linear increase ($P < 0.05$) in ADFI, BW, and ADG with increasing GZ dose. Quadratic GZ responses ($P < 0.05$) were observed for ADFI and ADG (phase 2 and 4 and overall) and day 41 BW. A linear increase or decrease in G:F ($P < 0.01$) occurred from GZ during phase 2 or phase 4, respectively, and no G:F difference ($P > 0.05$; orthogonal or LS mean) was observed during phase 3 or overall through day 41. Other than phase 3 ADFI and ADG, pigs fed GZ had higher ($P > 0.05$) ADFI, BW, and ADG than the NC-fed pigs during each phase and overall. Pigs fed 4,000 FTU per kg phytase had the highest ADFI, BW, and ADG throughout the trial and were statistically greater ($P < 0.05$) than ≤1,000 FTU per kg phytase- and PC-fed pigs, overall through day 41.

Pigs fed the PC treatment had greater ($P < 0.0001$; Table 4) percent and weight of bone ash, BBS, and apparent P digestibility than the pigs fed the nonsupplemented NC treatment. Linear and quadratic increases ($P < 0.001$) in percent and weight of bone ash, BBS, and apparent P digestibility were observed with GZ inclusion. Pigs fed ≥500 FTU per kg phytase had higher ($P < 0.0001$) apparent P digestibility than both NC- and PC-fed pigs. Feeding ≥500 FTU per kg phytase resulted in higher ($P < 0.0001$) percent and weight of bone ash and BBS than the NC treatment. Pigs fed ≥1,000 or ≥2,000 FTU per kg phytase had equivalent ($P > 0.05$) BBS or percent bone ash, respectively, as PC-fed pigs. For weight of bone ash, pigs fed

Table 3. Effect of phytase on nursery pig performance by growth phase and overall

			GraINzyme phytase, FTU[1] per kg					*P*-value			Sex effects			
Item, kg	PC[2]	NC[2]	500	1,000	2,000	4,000	SEM	Treatment	Linear[3]	Quadratic[3]	Barrows	Gilts	SEM	*P*-value
Phase 2: day 1 to 14 on treatment														
BW day 1	6.58	6.56	6.57	6.58	6.55	6.56	0.06	0.9992	0.9120	0.9346	6.70^{a}	6.43^{b}	0.03	<0.0001
BW day 14	10.15^{a}	9.63^{b}	10.18^{a}	10.29^{a}	10.39^{a}	10.51^{a}	0.17	0.0163	0.0033	0.0523	10.40^{a}	9.98^{b}	0.10	0.0073
ADG	0.255^{a}	0.219^{b}	0.258^{a}	0.266^{a}	0.275^{a}	0.282^{a}	0.011	0.0034	0.0008	0.0247	0.265	0.254	0.006	0.2361
ADFI	0.317^{a}	0.281^{b}	0.327^{a}	0.325^{a}	0.330^{a}	0.328^{a}	0.010	0.0076	0.0212	0.0109	0.321	0.315	0.006	0.4778
G:F	0.804	0.779	0.787	0.820	0.834	0.860	0.022	0.0985	0.0050	0.4540	0.824	0.803	0.013	0.2621
Phase 3: day 15 to 28 on treatment														
BW day 28	15.89^{ab}	14.47^{c}	15.34^{b}	15.89^{ab}	15.95^{ab}	16.67^{a}	0.31	0.0003	<0.0001	0.0981	15.88	15.52	0.19	0.1786
ADG	0.405^{ab}	0.340^{c}	0.362^{bc}	0.394^{ab}	0.397^{ab}	0.433^{a}	0.017	0.0071	0.0003	0.3310	0.386	0.392	0.010	0.6807
ADFI	0.609^{bcd}	0.538^{d}	0.581^{cd}	0.629^{b}	0.625^{b}	0.682^{a}	0.014	<0.0001	<0.0001	0.0543	0.609	0.612	0.008	0.8300
G:F	0.666	0.631	0.608	0.627	0.635	0.634	0.023	0.6278	0.6077	0.9709	0.630	0.637	0.014	0.6987
Phase 4: day 29 to 41 on treatment														
BW day 41	26.01^{b}	22.93^{c}	25.06^{b}	25.91^{b}	26.08^{ab}	27.23^{a}	0.41	<0.0001	<0.0001	0.0039	26.05^{a}	25.02^{b}	0.25	0.0052
ADG	0.778^{ab}	0.651^{c}	0.736^{b}	0.770^{ab}	0.779^{ab}	0.812^{a}	0.015	<0.0001	<0.0001	0.0006	0.778^{a}	0.731^{b}	0.009	0.0008
ADFI	1.156^{bc}	0.925^{d}	1.116^{c}	1.188^{bc}	1.215^{b}	1.331^{a}	0.031	<0.0001	<0.0001	0.0013	1.186^{a}	1.125^{b}	0.019	0.0263
G:F	0.627^{ab}	0.653^{ab}	0.610^{abc}	0.605^{bc}	0.594^{bc}	0.567^{c}	0.016	0.0111	0.0009	0.2640	0.613	0.605	0.010	0.5438
Overall: day 1 to 41 on treatment														
ADG	0.474^{b}	0.399^{c}	0.451^{b}	0.472^{b}	0.476^{ab}	0.504^{a}	0.010	<0.0001	<0.0001	0.0034	0.472^{a}	0.453^{b}	0.006	0.0320
ADFI	0.683^{bc}	0.573^{d}	0.664^{c}	0.703^{bc}	0.711^{b}	0.767^{a}	0.014	<0.0001	<0.0001	0.0004	0.694	0.673	0.008	0.0867
G:F	0.665	0.667	0.640	0.644	0.643	0.628	0.015	0.3680	0.1269	0.6481	0.651	0.645	0.009	0.6233

[a–d]Within a row, least square means without a common superscript differ ($P < 0.05$).

[1]FTU = GraINzyme (Agrivida Inc., Woburn, MA) phytase units, added to NC diet.

[2]PC = positive control; NC = negative control (−0.15% P).

[3]Orthogonal contrast of adding phytase to NC diet.

Table 4. Effect of phytase on apparent P digestibility, bone breaking strength (BBS), and bone ash weight (AshWt) and percentage (PAsh) of nursery pigs

			GraINzyme phytase, FTU[1] per kg					*P*-value			Sex effects			
Item, kg	PC[2]	NC[2]	500	1,000	2,000	4,000	SEM	Treatment	Linear[3]	Quadratic[3]	Barrows	Gilts	SEM	*P*-value
Apparent P digestibility (Pdig; day 35)														
Pdig, %	40.32[d]	33.19[e]	57.27[c]	57.24[c]	65.45[b]	72.75[a]	2.40	<0.0001	<0.0001	<0.0001	56.06	52.68	1.47	0.1125
Metacarpal bone parameters (day 41)														
BBS, kg	44.62[a]	31.84[c]	38.96[b]	45.43[a]	43.50[ab]	43.98[ab]	1.89	<0.0001	0.0005	0.0005	41.99	40.79	1.14	0.4599
AshWt, g	1.55[b]	1.11[d]	1.40[c]	1.50[b]	1.50[b]	1.69[a]	0.03	<0.0001	<0.0001	<0.0001	1.50[a]	1.42[b]	0.02	0.0108
PAsh, %	46.38[ab]	40.67[d]	44.94[c]	44.78[c]	45.80[bc]	47.18[a]	0.44	<0.0001	<0.0001	<0.0001	44.67	45.25	0.26	0.1255

[a–e]Within a row, least square means without a common superscript differ (*P* < 0.05).

[1]FTU = GraINzyme (Agrivida Inc., Woburn, MA) phytase units, added to NC diet.

[2]PC = positive control; NC = negative control (−0.15% P).

[3]Orthogonal contrast of adding phytase to NC diet.

1,000 or 2,000 FTU per kg phytase were equivalent (*P* > 0.05) to PC-fed pigs, where pigs fed 4,000 FTU per kg phytase were higher than all other treatments (*P* < 0.0001).

DISCUSSION

An increased feed intake and subsequent body weight gain in barrows compared to gilts is typically more noticeable in growing vs. starting pigs (Wolter and Ellis, 2001; Hinson et al., 2009). In the current trial, an increase in ADG was observed in barrows vs. gilts during the last phase and overall; however, the barrows were heavier than gilts at the initiation of the trial.

In the current trial, ADG and ADFI linearly increased by increasing GZ from 500 to 4,000 FTU per kg phytase. Previous research with a corn-expressed *E. coli* phytase (Nyannor et al., 2007) observed a linear increase in ADG and G:F from feeding 16,500, 33,000, and 49,500 FTU per kg to 10-kg pigs for 28 d. These authors reported that the *E. coli* phytase, whether produced microbially or expressed corn, responded similar when 16,500 FTU per kg was fed. In the current trial, there was a linear improvement in 14-d G:F as GZ increased; however, no treatment differences were observed in G:F through 41 d. Kühn and Partanen (2012) reduced Ca by 0.16% and P by 0.1% and reported a similar G:F response. The authors reported that G:F increased with *Trichoderma reesei* produced phytase addition through day 25 but not statistically different through day 46; however, 1,000 FTU per kg did not respond better than 500 FTU per kg. Veum et al. (2006) reduced only P (by 0.15%; as in current study) and did not see difference between PC and NC in 14-d ADG but a significant difference in overall 28-d ADG and 14-d and 28-d G:F was observed with PC diet. In that research, 500 FTU per kg of *E. coli* phytase improved G:F but not ADG, comparable to PC, where 2,500 and 12,500 FTU per kg responded better in both ADG and G:F than PC during the 14 to 28-d period. In the current trial, 500 FTU per kg phytase from GZ had equivalent ADG as PC through all periods and 4,000 FTU per kg phytase from GZ had greater overall (1 to 41 d) ADG than PC treatment.

In the current trial, feeding 4,000 FTU per kg GZ resulted in statistically greater bone ash (weight and percentage) than 2,000 FTU per kg dose and feeding 500 FTU per kg phytase, or higher, was greater than NC-fed pigs. Reduced bone ash has been previously reported by Braña et al. (2006) when nursery pigs were fed diets with 0.1% P reduction and that 750 FTU per kg of *E. coli* phytase was required to get statistically higher bone ash percentage as compared to the nonsupplemented, low-P control treatment. However, these researchers reported pigs fed 1,000 FTU per kg phytase had equivalent bone ash as the those fed 10,000 FTU per kg phytase treatment and feeding 500 FTU per kg or higher were equivalent to PC. Other researchers (Veum et al., 2006) demonstrated that greater than 500 FTU per kg was required, when reducing P by 0.15%, to acquire bone ash weight and strength equivalency to the PC treatment. In the current trial, feeding 1,000 or 2,000 FTU per kg phytase from GZ resulted in equivalent bone ash weight and bone strength as the PC-fed pigs.

Increasing GZ dose resulted in a linear increase in fecal P digestibility, as previously observed in weaned pigs fed a commercial phytase (Taylor et al., 2016). Previous research (Rutherfurd et al., 2014) has also demonstrated that the addition of *Aspergillus oryzae* phytase (1,105 or 2,215 U per kg) increased P digestibility over both the PC and

NC treatments. Other researchers (Bento et al., 2012; Torrallardona et al., 2012; Zeng et al., 2016) observed increased P digestibility with PC vs. NC diets and little or no difference in P digestibility between standard industry phytase levels (500 and 1,000 FTU per kg), similar to the current study with GZ. Further increases in P digestibility have been more notable when feeding doses of phytase at or above the superdosing levels: 2,000 or 4,000 FTU per kg in both the current study with GZ and previously with *A. oryzae* phytase (Torrallardona et al., 2012) or 20,000 FTU per kg of *E. coli* phytase (Zeng et al., 2014).

The improvements in average daily gain, P digestibility, and bone characteristics when GZ was supplemented to a low-P diet demonstrate that the Phy02 phytase within GZ is efficacious. The linear bone ash, P digestibility, and ADG responses observed, with 4,000 FTU per kg phytase being statistically highest, could imply these parameters may continue to improve with even higher phytase dose. The results of this study support the hypothesis that reducing only dietary P may increase phytase requirement. However, another study would need to be conducted including second set of phytase treatments, with both Ca and P being reduced, for complete confirmation of the hypothesis.

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