Journal of Animal Science, 2021, Vol. 99, No. 3, 1–11

doi:10.1093/jas/skab057
Advance Access publication February 24, 2021
Received: 21 November 2020 and **Accepted:** 18 February 2021
Non Ruminant Nutrition

NON RUMINANT NUTRITION

Formulating diets based on digestible calcium instead of total calcium does not affect growth performance or carcass characteristics, but microbial phytase ameliorates bone resorption caused by low calcium in diets fed to pigs from 11 to 130 kg

L. Vanessa Lagos,[†] Su A. Lee,[‡] Mike R. Bedford,[‖] and Hans H. Stein[†,‡,1]

[†]Division of Nutritional Sciences, University of Illinois, Urbana 61801, IL, USA, [‡]Department of Animal Sciences, University of Illinois, Urbana 61801, IL, USA, [‖]AB Vista, Marlborough SN8 4AN, UK

[1]Corresponding author: hstein@illinois.edu

Abstract

An experiment was conducted to test the hypothesis that the requirement for Ca expressed as a ratio between standardized total tract digestible (**STTD**) Ca and STTD P obtained in short-term experiments may be applied to pigs fed diets without or with microbial phytase from 11 to 130 kg. In a 5-phase program, 160 pigs (body weight: 11.2 ± 1.8 kg) were randomly allotted to 32 pens and 4 corn–soybean meal-based diets in a 2 × 2 factorial design with 2 diet formulation principles (total Ca or STTD Ca), and 2 phytase inclusion levels (0 or 500 units/kg of feed) assuming phytase released 0.11% STTD P and 0.16% total Ca. The STTD Ca:STTD P ratios were 1.40:1, 1.35:1, 1.25:1, 1.18:1, and 1.10:1 for phases 1 to 5, and STTD P was at the requirement. Weights of pigs and feed left in feeders were recorded at the end of each phase. At the conclusion of phase 1 (day 24), 1 pig per pen was euthanized and a blood sample and the right femur were collected. At the end of phases 2 to 5, a blood sample was collected from the same pig in each pen. At the conclusion of the experiment (day 126), the right femur of 1 pig per pen was collected and carcass characteristics from this pig were measured. No interactions were observed between diet formulation principle and phytase inclusion for growth performance in any phase and no differences among treatments were observed for overall growth performance. Plasma Ca and P and bone ash at the end of phase 1 were also not influenced by dietary treatments. However, on day 126, pigs fed nonphytase diets formulated based on total Ca had greater bone ash than pigs fed STTD Ca-based diets, but if phytase was used, no differences were observed between the 2 formulation principles (interaction $P < 0.05$). At the end of phases 2 and 3, pigs fed diets without phytase had greater ($P < 0.05$) plasma P than pigs fed diets with phytase, but no differences were observed at the end of phases 4 and 5. A negative quadratic effect ($P < 0.05$) of phase (2 to 5) on the concentration of plasma Ca was observed, whereas plasma P increased (quadratic; $P < 0.05$) from phases 2 to 5. However, there was no interaction or effect of diet formulation principle or phytase inclusion on any carcass characteristics measured. In conclusion, STTD Ca to STTD P ratios can be used in diet formulation for growing-finishing pigs without affecting growth performance or carcass characteristics and phytase inclusion ameliorates bone resorption caused by low dietary Ca and P.

Key words: bone ash, calcium, growth performance, phytase, pigs, phosphorus

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
AEE	acid hydrolyzed ether extract
BW	body weight
dCa	digestible Ca
FTU	phytase units per kilogram of feed
G:F	gain to feed ratio
HCW	hot carcass weight
LEA	loin eye area
LM	longissimus muscle
STTD	standardized total tract digestible
tCa	total Ca

Introduction

Values for standardized total tract digestible (**STTD**) Ca are believed to be additive in mixed diets for pigs (Stein et al., 2016). As a consequence, formulating diets based on values for STTD Ca in each ingredient instead of total Ca may increase accuracy of diet formulation (NRC, 2012). Recent work has generated values for the digestibility of Ca in most Ca-containing feed ingredients (Stein et al., 2016). Most STTD values were determined without and with inclusion of microbial phytase, because supplementation of exogenous phytase increases not only the digestibility of P but also the digestibility of Ca in some feed ingredients (González-Vega et al., 2013, 2015b).

The NRC (2012) indicated that requirements for Ca ideally would be expressed as a ratio between STTD Ca and STTD P, but because of a lack of data for the digestibility of Ca in commonly used feed ingredients at the time the NRC document was prepared, Ca requirements were expressed as requirements for total Ca (NRC, 2012). However, because data for the concentration of STTD Ca in feed ingredients are now available, the requirement for Ca can now be estimated based on STTD Ca:STTD P ratios. Data for Ca requirement of pigs from 11 to 22 kg (Lagos et al., 2019a), 25 to 50 kg (González-Vega et al., 2016), 50 to 85 kg (Lagos et al., 2019b), and 100 to 130 kg (Merriman et al., 2017) indicate that a ratio between STTD Ca and STTD P below 1.40:1, 1.35:1, 1.25:1, and 1.10:1, respectively, will maximize growth performance of pigs in these 4 weight groups. Results of these studies also demonstrated that the STTD Ca:STTD P ratios needed to maximize bone ash are greater than the ratios needed to maximize growth performance and ratios of 1.70:1, 1.80:1, 2.00:1, and 2.30:1 were determined to maximize bone ash in pigs from 11 to 22 kg, 25 to 50 kg, 50 to 85 kg, and 100 to 130 kg, respectively. These experiments were conducted over 3 to 5 wk, and therefore, a follow-up experiment through the entire period from 11 to 130 kg was needed to confirm that the ratios established to optimize growth performance within each weight group will also optimize growth performance throughout the growing-finishing phases. Therefore, the objective of this experiment was to test the hypothesis that the requirement for Ca expressed as a ratio between STTD Ca and STTD P by growing pigs obtained in short-term experiments may be applied to pigs from 11 to 130 kg without detrimental effects on growth performance. The second hypothesis was that pigs fed diets based on values for STTD Ca will have growth performance that is not different from that of pigs fed diets formulated based on total Ca.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment. Pigs used in the experiment were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN).

Animal and housing

One hundred and sixty pigs with an initial average body weight (**BW**) of 11.2 ± 1.8 kg were allotted to 4 diets in a completely randomized design on day 18 postweaning. There were 5 pigs per pen (3 gilts and 2 castrates) and 8 replicate pens per diet. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets. During the nursery phase (phase 1), pigs were housed in pens that had fully slatted floors, a feeder, and a nipple drinker. On day 24, pigs had an average BW of 26.8 ± 3.1 kg and were moved to a mechanically ventilated grower-finisher unit, where pens had partly slatted concrete floors and were equipped with a feeder and a nipple drinker. Feed and water were available at all times.

Diets and feeding

From weaning on day 20 to day 17 postweaning, pigs were fed a common diet that met all nutrient requirements for pigs from 5 to 7 kg (NRC, 2012). From day 18 postweaning (day 1 of the experiment), a 5-phase program was used (11 to 25 kg, 25 to 50 kg, 50 to 75 kg, 75 to 100 kg, and 100 to 135 kg). Phase changes were determined based on average pig weights and all pens changed phase on the same day. Thus, phases 1 to 4 were concluded at days 24, 52, 77, and 101, when pigs had a weight close to 25, 50, 75, and 100 kg, respectively. The experiment was terminated on day 126. In each phase, 4 diets based on corn and soybean meal (Table 1) were formulated for a total of 20 diets in the 5 phases (Tables 2 and 3). Within each phase, diets were formulated using a 2 × 2 factorial design with 2 requirement estimates for Ca (total Ca or STTD Ca), and 2 inclusion levels of microbial phytase [0 or 500 phytase units/kg of feed (**FTU**)]. In each phase, 1 diet was formulated based on the NRC (2012) requirement for total Ca (0.70%, 0.66%, 0.59%, 0.52%, and 0.46% for phases 1 to 5, respectively) and STTD P (0.33%, 0.31%, 0.27%, 0.24%, and 0.21% for phases 1 to 5, respectively). The second diet within each phase was formulated based on a ratio between STTD Ca and STTD P of 1.40:1, 1.35:1, 1.25:1, 1.18:1, and 1.10:1 for phases 1, 2, 3, 4, and 5, respectively (González-Vega et al., 2016; Merriman et al., 2017; Lagos et al., 2019a, 2019b). Concentrations of STTD P

Table 1. Analyzed composition of ingredients, as fed basis

Item	Corn	Soybean meal	Calcium carbonate	Monocalcium phosphate
Gross energy, Mcal /kg	3.78	4.32	—	—
Dry matter, %	84.50	88.36	99.96	92.15
Ash, %	1.34	6.94	92.65	81.23
Crude protein, %	7.25	49.84	—	—
AEE,[1] %	2.67	0.92	—	—
Ca, %	0.03	0.35	38.96	17.70
P, %	0.30	0.78	0.04	20.91
Phytate-bound P, %	0.13	0.45	—	—
Phytate,[2] %	0.46	1.60	—	—
Non-phytate P,[3] %	0.17	0.33	—	—

[1]AEE = acid hydrolyzed ether extract.
[2]Phytate was calculated by dividing phytate-bound P by 0.282 (Tran and Sauvant, 2004).
[3]Non-phytate P was calculated as the difference between total P and phytate-bound P.

Table 2. Ingredient composition and calculated and analyzed values of experimental diets formulated based on total Ca (tCa) or STTD Ca (dCa), without microbial phytase or with 500 FTU (phases 1, 2, and 3)[1]

Item	Phase 1				Phase 2				Phase 3			
Phytase inclusion:	0 FTU		500 FTU		0 FTU		500 FTU		0 FTU		500 FTU	
Ca requirement:	tCa	dCa	tCa	dCa	tCa	dCa	tCa	dCa	tCa	dCa	tCa	dCa
Ingredient, %												
Ground corn	52.02	52.42	53.48	53.32	72.25	72.76	73.76	73.63	76.22	76.89	77.68	77.74
Soybean meal, 48% CP[2]	32.00	32.00	32.00	32.00	22.00	22.00	22.00	22.00	18.50	18.50	18.50	18.50
Lactose	10.00	10.00	10.00	10.00	—	—	—	—	—	—	—	—
Choice white grease	2.40	2.20	1.68	1.75	2.58	2.32	1.82	1.90	2.50	2.18	1.78	1.75
Calcium carbonate	1.10	0.90	0.95	1.04	1.09	0.84	0.94	0.99	1.00	0.65	0.86	0.83
Monocalcium phosphate	0.94	0.94	0.34	0.34	0.93	0.93	0.32	0.32	0.76	0.76	0.15	0.15
Sodium bicarbonate	0.35	0.35	0.35	0.35	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Lys HCl, 78% Lys	0.37	0.37	0.37	0.37	0.34	0.34	0.34	0.34	0.28	0.28	0.28	0.28
DL-Met	0.15	0.15	0.15	0.15	0.07	0.07	0.07	0.07	0.03	0.03	0.03	0.03
L-Thr	0.12	0.12	0.12	0.12	0.09	0.09	0.09	0.09	0.06	0.06	0.06	0.06
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[3]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Phytase concentrate[4]	—	—	0.01	0.01	—	—	0.01	0.01	—	—	0.01	0.01
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated values												
Ca, %	0.70	0.62	0.54	0.57	0.66	0.57	0.50	0.52	0.59	0.45	0.43	0.42
P, %	0.56	0.56	0.44	0.44	0.54	0.54	0.42	0.42	0.49	0.49	0.36	0.37
STTD Ca, %	0.52	0.46	0.39	0.41	0.49	0.42	0.36	0.37	0.43	0.34	0.30	0.30
STTD P, %	0.33	0.33	0.22	0.22	0.31	0.31	0.20	0.20	0.27	0.27	0.16	0.16
STTD Ca:STTD P	1.56	1.40	1.32	1.40	1.57	1.35	1.30	1.35	1.59	1.25	1.28	1.25
Analyzed values												
Gross energy, Mcal/kg	3.96	4.00	3.97	3.99	3.96	3.98	3.91	3.93	3.95	3.95	3.95	3.93
Dry matter, %	88.77	88.47	88.41	88.27	91.09	89.67	89.59	89.44	88.12	87.91	87.93	87.87
Ash,%	5.18	4.64	4.49	4.57	4.17	4.08	3.81	3.88	4.35	3.83	3.64	3.74
Crude protein, %	21.83	19.47	18.97	18.77	16.81	17.14	16.80	18.36	14.72	15.26	14.59	13.88
AEE,[2] %	4.39	3.73	3.19	3.19	4.65	4.19	3.64	3.91	5.29	4.52	4.37	4.40
Ca, %	0.73	0.64	0.53	0.59	0.65	0.64	0.57	0.57	0.61	0.48	0.46	0.47
Total P, %	0.62	0.59	0.44	0.44	0.59	0.60	0.44	0.45	0.52	0.53	0.38	0.39
Phytate-bound P, %	0.19	0.16	0.17	0.16	0.26	0.25	0.25	0.25	0.25	0.24	0.25	0.25
Phytate,[5] %	0.67	0.57	0.60	0.57	0.92	0.89	0.89	0.89	0.89	0.85	0.89	0.89
Non-phytate P,[6] %	0.43	0.43	0.27	0.28	0.33	0.35	0.19	0.20	0.27	0.29	0.13	0.14
Phytase activity, FTU	<50	<50	572	640	<50	<50	509	569	<50	<50	567	464

[1]Diets were formulated to have the following quantities of net energy and amino acids (standardized ileal digestible amino acids): net energy: 2,479, 2,528, and 2,557 kcal per kilogram; Lys: 1.23, 0.98, and 0.85%; Met: 0.42, 0.31, and 0.25%; Thr: 0.74, 0.59, and 0.52%; and Trp: 0.22, 0.17, and 0.15% for phases 1, 2, and 3, respectively.
[2]AEE = acid hydrolyzed ether extract.
[3]The vitamin–micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2,210 IU; vitamin E as $_{DL}$-α tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin,6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; $_{D}$pantothenic acid as $_{D}$calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 125 mg as ironsulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc sulfate.
[4]The phytase concentrate contained 5,000 FTU per g (Quantum Blue, AB Vista, Marlborough, UK).
[5]Phytate was calculated by dividing the phytate-bound P by 0.282 (Tran and Sauvant, 2004).
[6]Non-phytate P was calculated as the difference between total P and phytate-bound P.

in these diets were based on NRC (2012), whereas concentrations of Ca corresponded to values of 0.62%, 0.57%, 0.45%, 0.38%, and 0.31% total Ca for phases 1 to 5, respectively. Thus diets formulated to meet specific ratios between STTD Ca and STTD P contained less total Ca than diets formulated to meet the NRC (2012) requirement for total Ca. Values for STTD Ca used in the formulation of these diets were obtained for each Ca-containing ingredient in the absence of phytase (Stein et al., 2016). The third diet within each phase was formulated as the first diet with the exception that 500 FTU of microbial phytase (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK) were included, and the provisions of total Ca and STTD P were reduced by 0.16 and 0.11 percentage units, respectively, compared with requirement estimates (NRC, 2012) to account for the expected release of Ca and P as a result of phytase inclusion. The last diet in each phase also contained microbial phytase (500 FTU) and the provision of STTD P was reduced by 0.11% compared with the NRC (2012) requirement. However, Ca was included to meet a ratio between STTD Ca and STTD P of 1.40:1, 1.35:1, 1.25:1, 1.18:1, and 1.10:1 for phases 1, 2, 3, 4, and 5, respectively, and STTD Ca values for each ingredient were based on values that were determined in the presence of phytase (Stein et al., 2016) to account for the increased STTD of Ca in some ingredients that is the result of phytase addition. Concentrations of Ca corresponded to values

Table 3. Ingredient composition and calculated and analyzed values of experimental diets formulated based on total Ca (tCa) or STTD Ca (dCa), without microbial phytase or with 500 FTU (phases 4 and 5)[1]

Item	Phase 4				Phase 5			
Phytase inclusion:	0 FTU		500 FTU		0 FTU		500 FTU	
Ca requirement:	tCa	dCa	tCa	dCa	tCa	dCa	tCa	dCa
Ingredient, %								
Ground corn	84.87	85.61	86.37	86.40	89.11	89.89	90.57	90.66
Soybean meal, 48% CP[2]	11.00	11.00	11.00	11.00	7.00	7.00	7.00	7.00
Choice white grease	1.36	0.98	0.60	0.58	1.35	0.95	0.62	0.56
Calcium carbonate	0.91	0.55	0.76	0.75	0.83	0.45	0.68	0.65
Monocalcium phosphate	0.70	0.70	0.10	0.10	0.59	0.59	—	—
Sodium bicarbonate	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Lys HCl, 78% Lys	0.36	0.36	0.36	0.36	0.33	0.33	0.33	0.33
DL-Met	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01
L-Thr	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Trp	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.03
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin–mineral premix[3]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Phytase concentrate[4]	—	—	0.01	0.01	—	—	0.01	0.01
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated values								
Ca, %	0.52	0.38	0.36	0.35	0.46	0.31	0.30	0.29
P, %	0.45	0.45	0.32	0.32	0.41	0.41	0.29	0.29
STTD Ca, %	0.38	0.29	0.26	0.25	0.34	0.23	0.21	0.20
STTD P, %	0.24	0.24	0.13	0.13	0.21	0.21	0.10	0.10
STTD Ca:STTD P	1.58	1.18	1.21	1.18	1.59	1.10	1.14	1.10
Analyzed values								
Gross energy, Mcal/kg	3.82	3.82	3.84	3.84	3.82	3.84	3.81	3.82
Dry matter, %	87.54	87.57	87.85	86.91	87.32	87.04	86.95	87.04
Ash,%	4.02	3.19	3.12	2.82	3.31	3.14	3.00	2.70
Crude protein, %	11.21	10.99	11.39	11.14	9.77	9.12	10.19	9.23
AEE,[2] %	4.34	3.41	3.17	3.37	3.49	2.89	2.53	2.67
Ca, %	0.55	0.43	0.40	0.38	0.49	0.33	0.33	0.34
Total P, %	0.46	0.46	0.35	0.34	0.42	0.42	0.30	0.29
Phytate-bound P, %	0.24	0.24	0.24	0.24	0.22	0.22	0.22	0.23
Phytate,[5] %	0.85	0.85	0.85	0.85	0.78	0.78	0.78	0.82
Non-phytate P,[6] %	0.22	0.22	0.11	0.10	0.20	0.20	0.08	0.06
Phytase activity, FTU	<50	<50	429	469	<50	<50	575	659

[1]Diets were formulated to have the following quantities of net energy and amino acids (standardized ileal digestible amino acids): net energy: 2,570 and 2,599 kcal per kg; Lys: 0.73% and 0.61%; Met: 0.22% and 0.19%; Thr: 0.46% and 0.40%; and Trp: 0.13% and 0.11% for phases 4 and 5, respectively.
[2]AEE = ether hydrolyzed ether extract.
[3]The vitamin–micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2,210 IU; vitamin E as $_{DL-}$ α tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin,6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; $_{D-}$pantothenic acid as $_{D-}$calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 125 mg as ironsulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.
[4]The phytase concentrate contained 5,000 FTU per g (Quantum Blue, AB Vista, Marlborough, UK).
[5]Phytate was calculated by dividing the phytate-bound P by 0.282 (Tran and Sauvant, 2004).
[6]Non-phytate P was calculated as the difference between total P and phytate-bound P.

of 0.54%, 0.52%, 0.42%, 0.35%, and 0.29% total Ca for phases 1 to 5, respectively. In each phase, the 4 diets were formulated to contain the same quantities of net energy, Na, Cl, K, vitamin D, and all other nutrients.

Sample collection, carcass characteristics, and bone measurements

The amount of feed offered was recorded daily and the amount of feed in the feeders and the weight of the pigs were recorded at the conclusion of each phase. On the last day of phase 1, the gilt in each pen with a BW closest to the average BW of the pen was euthanized and a blood sample was collected in a lithium-heparin-containing tube and the right femur was also collected. Blood samples were immediately centrifuged and plasma was harvested and stored at –20 °C until analyzed. At the conclusion of phase 2, the gilt in each pen with a BW closest to the average BW of the pen was identified and a blood sample was collected by jugular venipuncture. Blood samples were collected from the same gilt on the last day of phases 3 and 4. On the morning of the last day of phase 5, the 32 gilts (1 per pen) that had been bled at the end of phases 2, 3, and 4 were transported to the Meat Sciences Laboratory at the University of Illinois (3 km) and kept

in lairage overnight with free access to water. On the morning of the following day, pigs were weighed and humanely slaughtered as described by Overholt et al. (2016). A blood sample and the left femur were collected from each pig and standard carcass measurements were determined after slaughter.

Hot carcass weight (**HCW**) was recorded ~45 min postmortem and carcass yield was calculated by dividing the HCW by the live weight obtained immediately before slaughter. Carcasses were split down the middle and stored at 4 °C for 24 hr. Left half-carcasses were separated between the 10th and 11th rib to access the longissimus muscle (**LM**). Back fat thickness was measured at the 10th rib at 75% of the distance of the LM from the dorsal side of the vertebral column. The loin eye area (**LEA**) was determined by tracing the surface of the LM on acetate paper and measuring the tracings in duplicate on a digitizer tablet (Wacom, Vancouver, WA). Carcass lean percentage was calculated using the equation developed by Burson and Berg (2001): carcass lean % = ([8.588 + (0.465 × HCW, lb) – (21.896 × 10th rib back fat, in) + (3.005 × 10th LEA, in^2)] ÷ HCW, lb) × 100. Loin quality was determined by measuring ultimate pH, instrumental color, drip loss, and subjective marbling, visual color (NPPC, 1999), and firmness scores (NPPC, 1991) in 3 cuts of the LM with ~2.5 cm of thickness that were collected from the posterior portion of each half-carcass. Ultimate pH was measured 24 hr postmortem in 1 chop using a handheld pH meter and a glass electrode (Meat Probes Inc., Topeka, KS). Subjective measurements (i.e., marbling, visual color, and firmness) were performed in the same chop by a single trained employee from the University of Illinois. Instrumental color was measured in another chop with a CR-400 chroma meter (Minolta Camera Co., Ltd., Osaka, Japan) using a D65 light source, 2° observer angle, and 8 mm aperture calibrated with a white tile. Drip loss was determined in the third chop as the weight difference after and before being suspended from a fish hook for 24 hr at 4 °C (Honikel, 1998).

Collected femurs were autoclaved at 125 °C for 55 min and the remaining muscle and fat tissues attached to the bone were removed. Femurs were broken, dried overnight at 105 °C, and soaked for 72 hr in petroleum ether while placed in a chemical hood to remove marrow and remaining fat. Defatted femurs were left for 24 hr in the chemical hood to allow the ether to fully evaporate. Femurs were then dried at 135 °C for 2 hr and ashed at 600 °C for 16 hr. The weight of the femurs was recorded before and after drying and ashing to obtain the amount of ash (grams per femur) and the percentage of ash of the defatted dried bone.

Sample analysis

Ingredient samples were collected at the feed mill immediately after mixing, whereas each diet sample was a composite of samples collected from 8 randomly chosen 25-kg bags. Samples were later ground and subsampled for nutrient analysis. All samples were analyzed in duplicate. Corn, soybean meal, calcium carbonate, monocalcium phosphate, diets, bone ash, and plasma samples were analyzed for Ca and P by inductively coupled plasma-optical emission spectrometry (Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007] at the University of Missouri, Columbus, MO. Phytate-bound P was analyzed in corn, soybean meal, and all diets using a Foss near-infrared spectrometer with the phytate-P levels predicted using AUNIR calibration standards (AB Vista, Plantation, FL). Phytase activity was analyzed in diets by the ELISA method using Quantiplate Kits for Quantum Blue (AB Vista). All other analyses were conducted at the Monogastric Nutrition Laboratory at the University of Illinois, Urbana-Champaign. Ingredients and diets were analyzed for dry matter by oven drying at 135 °C for 2 hr (Method 930.15; AOAC Int., 2007) and for ash by incineration at 600 °C for 2 hr (Method 942.05; AOAC Int., 2007). Corn, soybean meal, and diets were also analyzed for N (Method 990.03; AOAC Int., 2007) using a LECO FP628 (LECO Corp., Saint Joseph, MI) and crude protein was calculated as N × 6.25. These samples were also analyzed for gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL) and for acid hydrolyzed ether extract (Method 2003.06; AOAC Int., 2007) using an Ankom^{HCl} hydrolyser and an Ankom^{XT15} extractor (Ankom Technology, Macedon, NY).

Calculations and statistical analyses

The percentage of phytate in corn, soybean meal, and diets was calculated by dividing the analyzed phytate-bound P by 0.282 (Tran and Sauvant, 2004), and nonphytate P was calculated by subtracting the amount of phytate-bound P from total P. Average daily gain (**ADG**), average daily feed intake (**ADFI**), and average gain to feed ratio (**G:F**) were calculated for pigs fed each experimental diet. Concentrations of bone Ca and bone P in grams per femur were calculated by multiplying the total quantity of bone ash by the percentage of Ca and P in bone ash.

Data were analyzed using SAS (SAS Inst. Inc., Cary, NC). Assumptions of the model and normality of residuals were tested using INFLUENCE, PROC GPLOT, and PROC UNIVARIATE options of SAS. Data for growth performance, concentration and percentage of bone ash, bone Ca, and bone P, concentrations of Ca and P in plasma, and carcass characteristics were analyzed by phase using the PROC MIXED of SAS with pen as the experimental unit. The model included the main effects of diet formulation principle (total Ca or STTD Ca) and phytase inclusion (0 or 500 FTU), and the interaction between diet formulation principle and phytase inclusion. Least squares means were separated using the PDIFF option of SAS. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Blood samples in phases 2, 3, 4, and 5 were collected from the same gilt, therefore, an additional analysis was conducted to analyze the effect of phase on plasma concentrations of Ca and P. These data were analyzed as repeated measures with unstructured variance using the PROC MIXED and REPEATED options of SAS. The model included diet formulation principle, phytase inclusion, and phase as main effects, phase as the time effect, and pig as the experimental unit. If the effect of phase was significant ($P < 0.05$), contrast statements were used to determine linear and quadratic effects of phase on the concentrations of Ca and P in plasma.

Results

All pigs consumed their diets without apparent problems, but 3 pigs did not complete the experiment. One pig fed the diet formulated based on STTD Ca:STTD P and no phytase was euthanized in phase 2 due to lameness. One pig was removed from the experiment in phase 4 due to bad condition, and 1 pig in apparent good condition died in phase 5; both of these pigs were fed the diet formulated based on total Ca and phytase. Values for growth performance parameters in the pens where the removed pigs were housed were adjusted as previously explained (Lindemann and Kim, 2007; Lee et al., 2016). The remaining pigs completed the experiment with no apparent health problems.

For ADG, ADFI, and G:F, there was no effect of inclusion of phytase or diet formulation principle at any phase or for the overall experimental period (Table 4). Likewise, no interactions between phytase inclusion and diet formulation principle on growth performance parameters were observed. However in phase 4, pigs fed diets formulated based on a ratio between STTD Ca and STTD P tended ($P < 0.10$) to have greater ADG and G:F than pigs fed diets formulated based on total Ca.

At the end of phase 1, none of the main effects or the interaction between main effects were significant for concentration (grams per femur) or percentage of bone ash, bone Ca, or bone P (Table 5). However, at the end of phase 5, if no phytase was used, pigs fed diets formulated based on total Ca had greater bone ash (concentration and percentage) and concentration of bone Ca and P than pigs fed diets formulated based on STTD Ca, but if phytase was used, no differences were observed in bone characteristics between the 2 diet formulation principles (interaction $P < 0.05$). However, values for percentage of bone ash and concentration of bone ash, bone Ca, and bone P from pigs fed diets formulated based on total Ca and no phytase were not different from values from pigs fed diets formulated based on STTD Ca and phytase (interaction $P < 0.05$). For percentage of bone Ca and bone P, the interaction between diet formulation principle and phytase inclusion was not significant, and no effect of diet formulation principle or phytase inclusion was observed.

The concentration of Ca and P in plasma of pigs was not affected by diet formulation principle at the end of phases 1, 2, 4, or 5 and no interaction between diet formulation principle and phytase inclusion was observed (Table 6). Likewise, there was no effect of diet formulation principle on plasma Ca at the end of phase 3, but for plasma P, an interaction ($P < 0.05$) between diet formulation principle and phytase inclusion was observed. There was no effect of diet formulation principle on pigs fed diets without phytase, but pigs fed diets with phytase had a greater ($P < 0.05$) concentration of plasma P at the end of phase 3 if diets were formulated based on STTD Ca than if diets were formulated based on total Ca. No effect of phytase inclusion on plasma Ca and P at the end of phases 1 and 4 was observed. Likewise, at the end of phases 2 and 3, plasma Ca was not affected by the inclusion of phytase, but at the end of phase 2, plasma P was greater ($P < 0.05$) in pigs fed diets without phytase than in pigs fed diets with phytase. At the end of phase 5, there was a tendency ($P < 0.10$) for pigs fed diets with phytase to have reduced concentration of Ca in plasma compared with pigs fed diets without phytase, but no effect of phytase inclusion on the concentration of plasma P was observed. When the effect of phases (2 to 5) was included in the model, no effect of diet formulation principle or phytase inclusion on the concentration of Ca and P in plasma was observed (Table 7). However, there was a linear and quadratic reduction ($P < 0.05$) in plasma Ca

Table 4. Growth performance of pigs fed diets formulated based on total Ca (tCa) or STTD Ca (dCa), without microbial phytase or with 500 units of microbial FTU[1]

	0 FTU		500 FTU			*P*-value		
Item,[2] kg	tCa	dCa	tCa	dCa	SEM	Diet	Phytase	Diet × phytase
Phase 1, days 1 to 24								
Initial BW	11.173	11.181	11.169	11.189	0.646	0.982	0.998	0.993
ADG	0.656	0.661	0.660	0.626	0.023	0.545	0.500	0.405
ADFI	1.040	1.021	1.031	0.998	0.046	0.580	0.730	0.873
G:F	0.633	0.650	0.642	0.630	0.009	0.772	0.534	0.093
Final BW	26.916	27.050	26.996	26.211	1.157	0.780	0.745	0.694
Phase 2, days 24 to 52								
ADG	0.848	0.782	0.750	0.788	0.037	0.704	0.226	0.168
ADFI	1.643	1.541	1.500	1.553	0.086	0.780	0.455	0.375
G:F	0.517	0.509	0.505	0.510	0.009	0.901	0.596	0.484
Final BW	50.736	48.893	48.008	48.236	2.063	0.699	0.419	0.619
Phase 3, days 52 to 77								
ADG	1.095	1.086	1.051	1.038	0.038	0.783	0.230	0.953
ADFI	2.489	2.433	2.420	2.393	0.087	0.638	0.534	0.871
G:F	0.440	0.448	0.435	0.434	0.008	0.703	0.269	0.639
Final BW	78.100	76.203	74.438	74.181	2.734	0.697	0.308	0.766
Phase 4, days 77 to 101								
ADG	1.035	1.085	0.982	1.047	0.03	0.074	0.148	0.808
ADFI	2.978	3.103	2.935	2.983	0.10	0.373	0.401	0.694
G:F	0.348	0.350	0.335	0.351	0.01	0.094	0.242	0.170
Final BW	102.940	102.240	98.003	99.300	3.24	0.928	0.234	0.759
Phase 5, days 101 to 126								
ADG	1.047	1.057	1.065	1.115	0.030	0.315	0.203	0.510
ADFI	3.401	3.457	3.435	3.487	0.078	0.492	0.687	0.981
G:F	0.308	0.306	0.310	0.321	0.007	0.577	0.248	0.390
Final BW	129.100	128.340	125.290	127.180	3.490	0.873	0.482	0.708
Overall phase, days 1 to 126								
ADG	0.936	0.930	0.906	0.921	0.024	0.854	0.409	0.659
ADFI	2.299	2.276	2.250	2.270	0.067	0.978	0.684	0.750
G:F	0.408	0.409	0.403	0.406	0.004	0.640	0.432	0.815

[1]Data are least squares means of 8 observations.
[2]BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; G:F = gain to feed ratio.

Table 5. Data from bones collected at the end of phase 1 (day 24) and 5 (day 126) from pigs fed diets formulated based on total Ca (tCa) or STTD Ca (dCa), without microbial phytase or with 500 units of microbial FTU[1]

	0 FTU		500 FTU			P-value		
Item	tCa	dCa	tCa	dCa	SEM	Diet	Phytase	Diet × phytase
End of phase 1 (day 24)								
Bone ash, g	13.25	13.20	12.01	12.51	0.693	0.748	0.175	0.698
Bone Ca, g	5.03	5.06	4.59	4.77	0.274	0.700	0.194	0.798
Bone P, g	2.40	2.45	2.20	2.27	0.124	0.630	0.140	0.921
Bone ash, %	50.28	50.75	49.39	49.46	0.823	0.743	0.197	0.810
Bone Ca, %	38.49	38.83	38.64	38.59	0.318	0.657	0.890	0.544
Bone P, %	18.13	18.52	18.29	18.14	0.138	0.392	0.427	0.058
End of phase 5 (day 126)								
Bone ash, g	81.07[a]	69.53[b]	70.62[b]	75.37[ab]	2.275	0.148	0.321	0.001
Bone Ca, g	30.47[a]	26.08[b]	26.35[b]	28.39[ab]	0.866	0.188	0.304	0.001
Bone P, g	14.40[a]	12.42[b]	12.53[b]	13.53[ab]	0.413	0.247	0.367	0.001
Bone ash, %	62.07[a]	60.15[b]	60.44[b]	60.99[ab]	0.394	0.092	0.327	0.004
Bone Ca[2], %	37.59	37.39	37.29	37.43	0.365	0.932	0.732	0.645
Bone P[2], %	17.77	17.80	17.74	17.87	0.161	0.594	0.911	0.762

[a,b]Means within a row lacking a common superscript letter are different ($P < 0.05$).
[1]Data are least squares means of 7 or 8 observations.

Table 6. Concentration of Ca and P in plasma of pigs fed diets formulated based on total Ca (tCa) or STTD Ca (dCa), without microbial phytase or with 500 units of microbial FTU[1]

	0 FTU		500 FTU			P-value		
Item, mg/dL	tCa	dCa	tCa	dCa	SEM	Diet	Phytase	Diet × phytase
End of phase 1, day 24								
Plasma Ca	10.55	9.95	10.15	9.92	0.267	0.131	0.428	0.489
Plasma P	10.66	11.71	10.77	10.22	0.492	0.615	0.169	0.117
End of phase 2, day 52								
Plasma Ca	10.50	10.56	10.64	10.54	0.176	0.922	0.735	0.664
Plasma P	12.01	12.54	10.66	10.84	0.458	0.449	0.002	0.707
End of phase 3, day 77								
Plasma Ca	10.34	9.93	10.23	10.36	0.206	0.509	0.451	0.208
Plasma P	12.23[a]	12.27[a]	10.70[b]	11.90[a]	0.209	0.006	< 0.001	0.011
End of phase 4, day 101								
Plasma Ca	10.64	10.11	10.36	10.28	0.217	0.172	0.810	0.295
Plasma P	12.38	11.81	12.25	11.83	0.371	0.194	0.881	0.854
End of phase 5, day 126								
Plasma Ca	9.81	9.40	9.28	9.25	0.168	0.207	0.059	0.274
Plasma P	10.76	11.26	11.32	11.08	0.338	0.703	0.569	0.282

[a,b]Means within a row lacking a common superscript letter are different ($P < 0.05$).
[1]Data are least squares means of 7 or 8 observations.

from phase 2 to phase 5. In contrast, a quadratic increase ($P < 0.05$) in plasma P was observed from phases 2 to 5.

There was no interaction between diet formulation principle and phytase inclusion for carcass characteristics, and no effect of diet formulation principle or phytase inclusion was observed (Table 8). However, carcasses from pigs fed diets formulated based on STTD Ca tended ($P < 0.10$) to have less marbling than carcasses from pigs fed diets formulated based on total Ca.

Discussion

Calcium is mainly supplied to pig diets by limestone, which is an inexpensive ingredient that sometimes is oversupplied in diets because it is often used as a carrier in vitamin–mineral premixes or as a flow agent in feed ingredients (Walk, 2016). However, excess dietary Ca reduces P digestibility in pigs (Stein et al., 2011; Velayudhan et al., 2019) and results in decreased feed intake and growth performance (Merriman et al., 2017; Lagos et al., 2019a). Therefore, Ca oversupply with limestone has negative effects on pig production. Microbial phytase is commonly used in swine diets to reduce inclusion of feed phosphates, which also results in reduced excretion of P in the manure (Jongbloed and Lenis, 1992). However, phytase not only increases P digestibility in plant feed ingredients (She et al., 2017) but also increases the digestibility of Ca in plant and animal feed ingredients and in limestone (González-Vega et al., 2013, 2015a; Lee et al., 2019), which may exacerbate the negative effect of excess Ca if the Ca-releasing effect of phytase is not taken into account in diet formulation. Therefore, requirements for Ca should be expressed as digestible Ca, and because pigs excrete endogenous Ca (González-Vega et al., 2013), the use of STTD values that are additive in mixed diets is more accurate than the use of apparent total tract digestible values (She et al., 2018). The STTD of Ca in most Ca sources used in pig diets have

Table 7. Concentration of Ca and P in plasma of pigs fed diets formulated based on total Ca (tCa) or STTD Ca (dCa), without microbial phytase or with 500 units of microbial FTU during phases 2, 3, 4, and 5[1]

	Diet		Phytase, FTU			P-value		Phase					P-value	
Item, mg/dL	tCa	dCa	0	500	SEM	Diet	Phytase	2	3	4	5	SEM	L	Q
Ca	10.22	10.06	10.19	10.09	0.076	0.161	0.336	10.56	10.23	10.35	9.43	0.096	<0.001	0.006
P	11.55	11.66	11.71	11.50	0.161	0.640	0.354	10.75	11.36	12.04	11.20	0.182	0.217	<0.001

[1]Data are least squares means of 60 or 61 observations for diet and phytase effects and 29 to 31 observations for phase effect.

Table 8. Carcass measurements of pigs fed diets formulated based on total Ca (tCa) or STTD Ca (dCa), without microbial phytase or with 500 units of microbial FTU[1]

	0 FTU		500 FTU			P-value		
Item	tCa	dCa	tCa	dCa	SEM	Diet	Phytase	Diet × phytase
HCW,[2] kg	96.87	94.91	89.18	93.33	3.903	0.782	0.245	0.441
Carcass yield, %	77.35	76.71	77.11	77.56	0.568	0.870	0.599	0.340
Backfat thickness, cm	1.53	1.57	1.51	1.48	0.131	0.959	0.675	0.781
LEA, cm^2	53.08	51.98	49.93	50.61	2.290	0.926	0.333	0.702
Carcass lean, %	56.13	55.93	56.19	55.90	0.732	0.739	0.987	0.947
Loin quality								
Ultimate pH	5.61	5.58	5.61	5.59	0.014	0.143	0.684	0.751
Visual color[3]	3.44	3.50	3.75	3.38	0.135	0.258	0.494	0.117
Marbling[4]	1.38	1.31	1.88	1.31	0.152	0.050	0.112	0.112
Firmness[5]	3.00	2.75	3.00	2.88	0.253	0.465	0.807	0.807
Instrumental color[6]								
L*	51.11	53.81	50.52	51.83	1.194	0.104	0.291	0.563
a*	8.69	8.82	8.49	9.16	0.658	0.550	0.919	0.686
b*	7.19	7.80	6.92	7.43	0.671	0.408	0.632	0.946
Drip loss, %	5.74	6.85	5.81	7.22	0.780	0.119	0.781	0.847

[1]Data are least squares means of 8 observations.
[2]HWC = hot carcass weight.
[3]Color score: 1 = pale pink to 6 = dark purplish red (NPPC, 1999).
[4]Marbling score: 1 = 1% intramuscular lipid to 10 = ≥10% intramuscular lipid (NPPC, 1999).
[5]Firmness score: 1 = very soft to 5 = very firm (NPPC, 1991).
[6]L* = lightness (the greater the value, the lighter the color), a* = redness (the greater the value, the redder the color), and b* = yellowness (the greater the value, the more yellow the color).

been reported (Stein et al., 2016), and experiments using pigs from 11 to 25 kg (Lagos et al., 2019a), 25 to 50 kg (González-Vega et al., 2016), 50 to 85 kg (Lagos et al., 2019b), and 100 to 130 kg (Merriman et al., 2017) have been conducted to determine the ratio between STTD Ca and STTD P that maximizes growth performance and bone mineralization. Therefore, in this experiment, the ratios that maximized growth performance of pigs in those 4 experiments were used to formulate diets without and with the inclusion of microbial phytase and these diets were fed to pigs from 11 to 130 kg. In each of the 4 short-term experiments, different dietary levels of Ca and P were used to determine the optimal ratio between STTD Ca and STTD P. However, in each experiment, the optimum dietary concentration for total Ca was also determined and it was concluded that as long as total Ca does not exceed NRC (2012) requirements, pig growth performance will not be reduced. It was therefore, expected that pigs fed diets formulated based on STTD Ca would obtain growth performance that was not different from that of pigs fed diets formulated based on total Ca provided that the concentration of total Ca did not exceed NRC (2012) requirements and results of the experiment confirmed this hypothesis.

One of the consequences of formulating diets based on STTD Ca is that the total Ca in the diet reflects the digestibility of Ca in ingredients, and if ingredients with lower STTD of Ca are used, a greater quantity of total Ca is needed to reach a certain level of STTD Ca in the diet. This is illustrated in Phase 1 and Phase 2 diets where the total Ca in diets containing phytase increased if diets were formulated based on STTD Ca because use of phytases reduces the need for monocalcium phosphate in the diets and more calcium carbonate, therefore, needs to be added. However, because the STTD of Ca in calcium carbonate is less than in monocalcium phosphate (González-Vega et al., 2015b), the concentration of total Ca in the diet will increase.

The lack of differences in growth performance of pigs among the 4 dietary treatments in each phase and for the entire experimental period indicates that regardless of the inclusion of phytase, both diet formulation principles can be used to formulate diets for growing-finishing pigs. These results also confirm that under the conditions of this experiment, the ratios between STTD Ca and STTD P obtained in short-term experiments can be used to optimize growth performance of pigs in the entire growing-finishing period. The observation that the growth performance of pigs fed diets with 500 FTU of phytase was not different between the 2 formulation principles was expected because the composition of these diets was almost identical. On the other hand, the observation that there are no differences in growth performance of pigs fed non-phytase diets based on total Ca or STTD Ca indicates that the reduced concentration of Ca in STTD Ca diets compared with diets based

on total Ca does not compromise growth performance of pigs. However, diets will be properly formulated only if the analyzed concentration of dietary Ca is consistent with the formulated value. Commercial diets from the swine and poultry industries in the European Union contain on average 0.22 percentage units more Ca than formulated (Walk, 2016), which negatively affects animal growth performance. Diets used in this experiment were formulated using a Ca-free vitamin–mineral premix and all Ca-containing ingredients contained Ca that was close to published values (NRC, 2012). The current data demonstrate that if Ca is not provided in excess of requirements, diets formulated based on requirements for total Ca (NRC, 2012) will result in the same growth performance of pigs as if diets are formulated based on a ratio between STTD Ca and STTD P. Thus, it is likely that avoiding excess Ca in the diets is at least as important as formulating diets based on STTD Ca and requirements for total Ca by NRC (2012) should be considered maximum values. In contrast, although not investigated in the present experiment, results of our previous work clearly indicate that NRC requirements for STTD P should be considered minimum requirements (Gonzalez-Vega et al., 2016; Merriman et al., 2017; Lagos et al., 2019a, 2019b).

Calcium and P requirements to maximize bone mineralization are greater than requirements to maximize growth performance of pigs (Crenshaw, 2001). Although this statement is true for all productive phases, the difference between requirements to optimize growth performance and bone ash is less in young pigs than in finishing pigs (Lagos et al., 2019a). This is likely the reason for the lack of differences in the concentration and percentage of Ca, P, and ash in bones from pigs fed nonphytase diets based on total Ca or STTD Ca during the first phase of the experiment. Without phytase, phase 1 diets based on STTD Ca were formulated to have 0.08 percentage units less Ca than diets formulated based on total Ca, and the observation that this did not affect bone mineralization of pigs indicates that the STTD Ca:STTD P ratio that maximizes growth performance of 11 to 25 kg does not negatively affect bone mineralization. The reason for this lack of differences is likely that these pigs had enough Ca stored from either the milk while nursing or from the common diet they were fed for 17 d after weaning. Around two-thirds of the defatted dry bone is composed of inorganic material, which mainly consists of calcium phosphate in the form of hydroxyapatite salts (Fails and Magee, 2018). The Ca to P ratio in the mineral portion of bones is maintained at 2.1:1 with concentrations of Ca and P in bone ash that range from 36% to 39% and from 17% to 19%, respectively (Crenshaw, 2001). The concentrations of Ca and P in bone ash obtained in this study are consistent with the values reported by Crenshaw (2001) and explain the lack of differences in the percentage of Ca and P in bone ash among treatments at the end of phase 5. However, because STTD Ca diets were formulated using ratios between STTD Ca and STTD P that maximize growth performance, the observation that without phytase, pigs fed diets based on STTD Ca had less bone ash than pigs fed total Ca diets was expected. The STTD Ca to STTD P ratio to optimize growth performance decreases from 1.40:1 to 1.10:1 as pigs get heavier (Lagos et al., 2019a), whereas the ratio between total Ca and STTD P recommended by NRC (2012) increases from 1.82:1 to 2.05:1 from phases 1 to 5. Thus, phase 2 diets based on STTD Ca were formulated to have 0.10 percentage units less Ca than total Ca diets, whereas for phase 5, diets were formulated to have a difference of 0.15 percentage units of Ca.

The interactions between diet formulation principle and phytase inclusion that were observed for bone characteristics at the end of phase 5 indicate that phytase ameliorates bone resorption caused by low Ca in diets formulated to meet specific ratios for STTD Ca to STTD P because bone ash (concentration and percentage) from pigs fed STTD Ca diets with phytase was not different from that from pigs fed total Ca diets without phytase. This observation also indicates that values for STTD Ca in feed ingredients with phytase that were used in diet formulation were accurate. The amount of ash in bone represents the bone size, whereas the percentage of bone ash represents the composition of the bone (Lagos et al., 2019b). Thus, the reason for the inconsistency in the results for growth performance (BW) and bone ash (grams) at the end of phase 5 is that only 1 pig per pen was used to analyze bone characteristics and this pig was chosen at the end of phase 2. Therefore, at the conclusion of the experiment, some of these pigs were above or below the average BW of the pen.

Finishing pigs have greater bone mineralization than young pigs as indicated by the difference in percentage of bone ash at the end of phases 1 and 5 (~50% and 60%, respectively). This observation is in agreement with a linear increase in bone ash (52% to 59%) observed from days 46 to 173 of age in pigs fed diets with different concentrations of Ca and P (Crenshaw et al., 1981). Values for percentage of bone ash obtained in this study concur with data from 24-kg pigs (49%; Lagos et al., 2019a) and 86-kg pigs (58%; Lagos et al., 2019b) fed diets with adequate levels of Ca and P. Bone ash in femur of parity 3 sows fed diets with 3 different ratios between Ca and P was on average 68% and 70% for pregnant/lactating and nonpregnant sows, respectively (Mahan and Fetter, 1982). These data indicate that the skeleton of young pigs has a greater proportion of organic components than older pigs. Data from monkeys support this hypothesis as indicated by an increase in the mineral content and the ratio between mineral and organic matter in bones from age 0 to 13 yr with a peak at 8 yr of age (Cerroni et al., 2000; Boskey and Coleman, 2010). Bone data in this experiment were only obtained from gilts because there is no effect of sex on bone development of growing-finishing pigs (Ganelang et al., 2014).

The concentration of plasma Ca in pigs was not affected by diet formulation principle or by phytase inclusion, which is likely a result of the hormonal regulation of Ca homeostasis (Veum, 2010). The concentration of Ca in plasma ranged from 9.25 to 10.64 mg/dL, which is within the physiological range of serum Ca in pigs (8 to 12 mg/dL; Amundson et al., 2017). Because P is less tightly regulated, a few differences were observed in the concentration of plasma P across dietary treatments at the end of phases 2 and 3. These data may indicate that during the early phases, P release by phytase was slightly overestimated and to meet the P requirement for bone mineralization, more P was pulled from the bloodstream to the bones, resulting in lower P concentration in plasma from pigs fed diets with phytase than from pigs fed non-phytase diets. However, this difference was not observed in phases 4 and 5. The negative quadratic effect of phase on the concentration of plasma Ca may be a result of the greater mineral content in bones of finishing pigs compared with young pigs. However, besides bone mineralization, P is also required for soft tissue deposition, therefore, the observation that there was a positive quadratic effect of phase on the concentration of P in plasma, may also reflect that older pigs deposit less lean tissue than young pigs. These results concur with observations that the ratio between STTD Ca and STTD P that maximizes bone mineralization increases as pigs grow, whereas the ratio that maximizes growth performance decreases as pigs grow (Lagos et al., 2019a).

The observation that carcass characteristics were not affected by formulation principle or phytase inclusion is in agreement with

observations that there are no differences in back fat thickness, LEA, or carcass yield among pigs fed diets with increasing ratios between Ca and P (Liu et al., 1998; Hanni et al., 2005). A reduction in slaughter BW and HCW of pigs fed diets with total Ca to total P ratios at or above 1.50:1, compared with pigs fed diets with a lower ratio was observed (Liu et al., 1998; Hanni et al., 2005), but in the present study, ratios between total Ca and total P did not exceed 1.25:1, which likely contributed to the lack of differences among treatments in carcass characteristics. Data from Liu et al. (1998) and Hanni et al. (2005) also demonstrate the negative effect of excess Ca on growth performance of pigs. Increased concentration of Ca in plasma and muscle improves the oxidative metabolism in muscle and results in increased meat quality (Wilborn et al., 2004). High doses of vitamin D_3 increase tenderness of beef cuts as a result of increased Ca mobilization, but when supplemented to pigs, color and ultimate pH, but not tenderness, of loin chops were improved (Wilborn et al., 2004). Diets used in this experiment had equal concentrations of vitamin D_3, which may have contributed to the lack of differences in loin quality measurements among dietary treatments. The observation that carcasses from pigs fed diets formulated on the basis of STTD of Ca tended to have reduced marbling compared with carcasses from pigs fed diets based on total Ca was not expected, and because the values for marbling obtained in this study are within a narrow range (1.31 to 1.88) it is difficult to hypothesize the reason behind this observation.

Conclusions

Ratios between STTD Ca and STTD P obtained in short-term experiments can be used to formulate diets without or with phytase for growing-finishing pigs without affecting growth performance parameters or carcass characteristics of pigs. However, results of the experiment demonstrated that as long as dietary Ca is not provided in excess of the requirement, diets may be formulated either based on values for STTD Ca or based on values for total Ca without negative impacts on pig growth performance. Inclusion of microbial phytase ameliorates bone resorption caused by low Ca in diets. The effect of formulation principle and phytase inclusion on plasma Ca and P was limited, but the effect of phase on the concentration of Ca and P in plasma reflects the changing needs for Ca and P for deposition of bone and soft tissue as pigs become older.

Acknowledgment

Financial support for this research from AB Vista, Marlborough, UK, is appreciated.

Conflict of Interest

M.R.B. is an employee at AB Vista, Marlborough, UK, which is a global supplier of microbial phytase. All other authors have no real or perceived conflicts of interest.

Literature Cited

Amundson, L. A., L. L. Hernandez, and T. D. Crenshaw. 2017. Serum and tissue 25-OH vitamin D_3 concentrations do not predict bone abnormalities and molecular markers of vitamin D metabolism in the hypovitaminosis D kyphotic pig model. *Br. J. Nutr.* **118**:30–40. doi:10.1017/S0007114517001751.

AOAC Int. 2007. *Official methods of analysis of AOAC int.* 18th Rev. 2. ed. Gaithersburg, MD: AOAC International.

Boskey, A. L., and R. Coleman. 2010. Aging and bone. *J. Dent. Res.* **89**:1333–1348. doi:10.1177/0022034510377791.

Burson, D., and E. Berg. 2001. *Procedures for estimating pork carcass composition. Pork facts*. Des Moines, IA: National Pork Producers Council.

Cerroni, A. M., G. A. Tomlinson, J. E. Turnquist, and M. D. Grynpas. 2000. Bone mineral density, osteopenia, and osteoporosis in the rhesus macaques of Cayo Santiago. *Am. J. Phys. Anthropol.* **113**:389–410. doi:10.1002/1096-8644(200011)113:3<389::AID-AJPA9>3.0.CO;2-I.

Crenshaw, T. D. 2001. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. In: A. J. Lewis, and L. L. Southern, editors, *Swine Nutrition*. 2nd ed. Boca Raton, FL: CRC Press; p. 187–212.

Crenshaw, T. D., E. R. Peo, Jr, A. J. Lewis, B. D. Moser, and D. Olson. 1981. Influence of age, sex and calcium and phosphorus levels on the mechanical properties of various bones in swine. *J. Anim. Sci.* **52**:1319–1329. doi:10.2527/jas1981.5261319x.

Fails, A. D., and C. Magee. 2018. *Anatomy and physiology of farm animals*. 8th ed. Hoboken, NJ: John Wiley & Sons; p. 91–92.

Ganelang, B., J. C. Moreki, C. M. Tsopito, and S. J. Nsoso. 2014. Effect of weaning age and sex on meat quality traits of pigs raised under intensive system and slaughtered at 70 kg body weight. *Online J. Anim. Feed Res.* **4**:113–120.

González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2013. Endogenous intestinal losses of calcium and true total tract digestibility of calcium in canola meal fed to growing pigs. *J. Anim. Sci.* **91**:4807–4816. doi:10.2527/jas.2013-6410.

González-Vega, J. C., C. L. Walk, M. R. Murphy, and H. H. Stein. 2016. Requirement for digestible calcium by 25 to 50 kg pigs at different dietary concentrations of phosphorus as indicated by growth performance, bone ash concentration, and calcium and phosphorus balances. *J. Anim. Sci.* **94**: 5272–5285. doi:10.2527/jas.2016-0751.

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015a. Effect of phytate, microbial phytase, fiber, and soybean oil on calculated values for apparent and standardized total tract digestibility of calcium and apparent total tract digestibility of phosphorus in fish meal fed to growing pigs. *J. Anim. Sci.* **93**:4808–4818. doi:10.2527/jas.2015-8992.

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015b. Effects of microbial phytase on apparent and standardized total tract digestibility of calcium in calcium supplements fed to growing pigs. *J. Anim. Sci.* **93**:2255–2264. doi:10.2527/jas.2014-8215.

Hanni, S. M., M. D. Tokach, R. D. Goodband, J. M. Derouchey, J. L. Nelssen, and S. S. Dritz. 2005. Effects of increasing calcium-to-phosphorus ratio in diets containing phytase on finishing pig growth performance. *Prof. Anim. Sci.* **21**:59–65. doi:10.15232/S1080-7446(15)31167-0

Honikel, K. O. 1998. Reference methods for the assessment of physical characteristics of meat. *Meat Sci.* **49**:447–457. doi:10.1016/s0309-1740(98)00034-5.

Jongbloed, A. W., and N. P. Lenis. 1992. Alteration of nutrition as a means to reduce environmental pollution by pigs. *Livest. Prod. Sci.* **31**:75–94. doi:10.1016/0301-6226(92)90057-B

Kim, B. G., and M. D. Lindemann. 2007. A new spreadsheet method for the experimental animal allotment. *J. Anim. Sci.* **85**(Suppl. 2):218 (Abstr.)

Lagos, L. V., S. A. Lee, G. Fondevila, C. L. Walk, M. R. Murphy, J. J. Loor, and H. H. Stein. 2019a. Influence of the concentration of dietary digestible calcium on growth performance, bone mineralization, plasma calcium, and abundance of genes involved in intestinal absorption of calcium in pigs from 11 to 22 kg fed diets with different concentrations of digestible phosphorus. *J. Anim. Sci. Biotechnol.* **10**:47. doi:10.1186/s40104-019-0349-2

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

Lee, S. A., C. Kong, O. Adeola, and B. G. Kim. 2016. Different coefficients and exponents for metabolic body weight in a model to estimate individual feed intake for growing-finishing pigs. *Asian-Australas. J. Anim. Sci.* **29**:1756–1760. doi:10.5713/ajas.16.0420.

Lee, S. A., L. V. Lagos, C. L. Walk, and H. H. Stein. 2019. Standardized total tract digestibility of calcium varies among sources of calcium carbonate, but not among sources of dicalcium phosphate, but microbial phytase increases calcium digestibility in calcium carbonate. *J. Anim. Sci.* **97**:3440–3450. doi:10.1093/jas/skz176

Lindemann, M. D., and B. G. Kim. 2007. Technical note: a model to estimate individual feed intake of swine in group feeding. *J. Anim. Sci.* **85**:972–975. doi:10.2527/jas.2006-412.

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

Mahan, D. C., and A. W. Fetter. 1982. Dietary Calcium and phosphorus levels for reproducing sows. *J. Anim. Sci.* **54**: 285–291. doi:10.2527/jas1982.542285x.

Merriman, L. A., C. L. Walk, M. R. Murphy, C. M. Parsons, and H. H. Stein. 2017. Inclusion of excess dietary calcium in diets for 100- to 130-kg growing pigs reduces feed intake and daily gain if dietary phosphorus is at or below the requirement. *J. Anim. Sci.* **95**:5439–5446. doi:10.2527/jas2017.1995.

NPPC. 1991. *Procedures to evaluate market hogs*. Des Moines, IA: National Pork Producers Council.

NPPC. 1999. *Official color and marbling standards*. Des Moines, IA: National Pork Producers Council.

NRC. 2012. *Nutrient requirements of swine*. 11th rev. ed. Washington, DC: National Academic Press.

Overholt, M. F., J. E. Lowell, E. K. Arkfeld, I. M. Grossman, H. H. Stein, A. C. Dilger, and D. D. Boler. 2016. Effects of pelleting diets without or with distillers' dried grains with solubles on growth performance, carcass characteristics, and gastrointestinal weights of growing-finishing barrows and gilts. *J. Anim. Sci.* **94**:2172–2183. doi:10.2527/jas.2015-0202.

She, Y., D. Li, and S. Zhang. 2017. Methodological aspects of determining phosphorus digestibility in swine: a review. *Anim. Nutr.* **3**:97–102. doi:10.1016/j.aninu.2017.02.003.

She, Y., Q. Wang, H. H. Stein, L. Liu, D. Li, and S. Zhang. 2018. Additivity of values for phosphorus digestibility in corn, soybean meal, and canola meal in diets fed to growing pigs. *Asian-Australas. J. Anim. Sci.* **31**:1301–1307. doi:10.5713/ajas.17.0547.

Stein, H. H., O. Adeola, G. L. Cromwell, S. W. Kim, D. C. Mahan, and P. S. Miller; North Central Coordinating Committee on Swine Nutrition (NCCC-42). 2011. Concentration of dietary calcium supplied by calcium carbonate does not affect the apparent total tract digestibility of calcium, but decreases digestibility of phosphorus by growing pigs. *J. Anim. Sci.* **89**:2139–2144. doi:10.2527/jas.2010-3522.

Stein, H. H., L. A. Merriman, and J. C. González-Vega. 2016. Establishing a digestible calcium requirement for pigs. In: C. L. Walk, I. Kühn, H. H. Stein, M. T. Kidd, and M. Rodehutscord, editors, *Phytate destruction - consequences for precision animal nutrition*. Wageningen, The Netherlands: Wageningen Academic Publishers; p. 207–216.

Tran, G., and D. Sauvant. 2004. Chemical data and nutritional value. In: D. Sauvant, J.-M. Perez, and G. Tran, editors, *Tables of composition and nutritional value of feed materials: pigs, poultry, cattle, sheep, goats, rabbits, horses and fish*. Wageningen, The Netherlands: Wageningen Academic Publishers; p. 17–24.

Velayudhan, D. E., L. Marchal, and Y. Dersjant-Li. 2019. Meta-analysis: effect of increasing dietary calcium and phytase dose on phosphorus digestibility in weaning piglets and growing pigs. *J. Anim. Sci.* **97** (Suppl. 3):353–354 (Abstr.). doi:10.1093/jas/skz258.704

Veum, T. L. 2010. Phosphorus and calcium nutrition and metabolism. In: D. M. S. S. Vitti, and E. Kebreab, editors, *Phosphorus and calcium utilization and requirements in farm animals*. Wallingford, UK: CABI; p. 94–111.

Walk, C. L. 2016. The influence of calcium on phytase efficacy in non-ruminant animals. *Anim. Prod. Sci.* **56**:1345–1349. doi:10.1071/AN15341

Wilborn, B. S., C. R. Kerth, W. F. Owsley, W. R. Jones, and L. T. Frobish. 2004. Improving pork quality by feeding supranutritional concentrations of vitamin D_3. *J. Anim. Sci.* **82**:218–224. doi:10.2527/2004.821218x.