

The impact of “super-dosing” phytase in pig diets on growth performance during the nursery and grow-out periods¹

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ABSTRACT: Previous research indicates that “super-dosing” phytase may improve pig growth performance by improved nutrient use, although the benefits appear to be more consistent in nursery than in grow-out pigs. Therefore, two experiments were conducted to determine if performance could be improved by feeding phytase at super-dosed levels, and whether this response would be different if energy and amino acid (AA) were limiting. Experiment 1 involved 440 weaned pigs (6.27 ± 0.01 kg) in a factorial arrangement of treatments comparing the main effects of diet (positive control [PC] balanced for all nutrients vs. a negative control [NC]: 10% lower standardized ileal digestible (SID) lysine with relative reduction of all other essential AA and 1% reduced fat) and phytase levels (0 vs. 2,500 FTU Quantum Blue 5G phytase/kg). Pigs were assigned to pen according to a randomized complete block design based on body weight (BW). Feed and water were provided ad libitum across four dietary phases: 3×1 wk plus 1×2 wk. The average daily gain (ADG) and gain to feed ratio (G:F) were improved in the PC relative to the NC ($P < 0.05$) indicating success in formulating a diet limiting in energy and/or AA. Phytase improved ADG and G:F, regardless of diet composition ($P < 0.05$). Thus, super-dosing

phytase improved nursery pig growth performance, irrespective of diet nutrient adequacy or deficit. Experiment 2 involved 2,200 growing pigs (36.6 ± 0.30 kg) allotted to five treatments: a balanced PC (250 FTU Quantum Blue 5G phytase/kg), an NC (PC with 15% less SID lysine and 1.5% lower net energy [NE]), and three super-dosing phytase treatments applied to the NC totaling 1,000, 1,750, and 2,500 FTU phytase/kg. Feed and water were available ad libitum. At trial completion (approximately 122 kg), the PC pigs were heavier and more efficient than the NC pigs ($P < 0.05$) indicating success in formulating an NC treatment. Super-dosing phytase had no effect on whole body ADG or average daily feed intake ($P > 0.10$) but tended to improve G:F and feed energy efficiency ($P < 0.10$). Super-dosing phytase improved carcass-based feed and feed energy efficiency ($P < 0.05$) and tended to improve ADG ($P < 0.10$). Supplying phytase at “super-dosed” levels—above that required to meet the phosphorus requirement—improved growth performance in nursery pigs (6 to 22 kg BW) and provided smaller benefits in grow-finish pigs (37 to 122 kg BW). The improvement during the nursery period was independent of energy and AA levels in the diet.

Key words: carcass growth, phytase, phytate, super-dosing phytase, swine

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¹Appreciation is expressed to the National Pork Board, AB Vista Feed Ingredients, and Vita Plus for financial support of this project and to Hanor Company for in-kind support. Appreciation is also expressed to Mike McGrath and Cate Zier-Rush for technical assistance.

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Received October 27, 2018.

Accepted December 27, 2018.

of *Animal Science*.

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Transl. Anim. Sci. 2019.3:420–428
doi: 10.1093/tas/txy148

INTRODUCTION

Inorganic phosphorus (P) is an expensive ingredient to include in pig diets. Furthermore, excess P in the diet, leading to excess P in the manure, can also have negative effects on the environment (Selle and Ravindran, 2007). Consequently, pig producers seek to maximize the utilization of naturally occurring P in basal diet ingredients. In plants, a considerable portion of P is stored as phytate, a complex structure with six phosphate molecules bound to a myo-inositol ring (Raboy, 2003). The exact proportion of P bound to phytate depends on the ingredient; for example, in cereal grains and oilseed meals, it ranges from 50% to 80% (NRC, 2012).

Phytase is an enzyme capable of dephosphorylating phytate. However, pigs do not produce phytase in their gastrointestinal tract in sufficient quantities to achieve significant destruction of phytate (Jongbloed et al., 1992; Eeckhout and De Paepe, 1994). Isolated yeast-derived or bacterially derived phytase is therefore added to pig diets to facilitate the release of phytate-bound P, providing environmental and financial benefits (Beaulieu et al., 2007; Rutherford et al., 2014).

“Super-dosing” is defined as using levels of phytase in great excess (3-fold or greater) of that required to fulfill the pig’s requirement for P, as a means to further improve pig performance (Humer et al., 2015), reduce total dietary phytate, and release lower inositol derivatives (Zier-Rush et al., 2012; Holloway, 2016). Results have been inconsistent. Zeng et al. (2014) reported improvements in weight gain and feed efficiency in nursery pigs, whereas Flohr et al. (2014) observed no benefits in grow-finish pigs. Therefore, the objective of these experiments was to determine if super-dosing phytase improves pig growth performance during the nursery and the grow-out phases of production. The overall hypothesis was that super-dosing phytase would improve pig performance and would be more effective during the nursery phase than the grow-out phase. A further objective during the nursery period was to determine if the response to super-dosed phytase would be magnified if energy

and amino acids (AAs) were present in the diet at less than optimal levels.

MATERIALS AND METHODS

The protocols were approved by the Institutional Animal Care and Use Committee of Iowa State University (1-14-7694-S and 9-14-7873-S, for experiment 1 and experiment 2, respectively).

Animals, Housing, and Experimental Design

Experiment 1. This experiment was conducted at the Swine Nutrition Farm, Iowa State University, Ames, IA. A total of 440 newly weaned pigs of mixed sex (PIC 337 × Tempel sows; PIC, Hendersonville, TN; Tempel Genetics Inc., Gentryville, IN) were received from a commercial sow unit, weighed, and randomly allotted to one of four treatments across 44 pens within 11 blocks based on initial body weight (BW; average BW: 6.27 ± 0.01 kg). Pens, each housing 10 pigs and providing 0.28 m² per pig, consisted of woven-wire floors, two individual nipple waterers, and a four-space, dry self-feeder providing ad libitum access to feed and water.

Pigs were vaccinated according to farm protocol with Circumvent PCV (Merck Animal Health, Summit, NJ) during the initial weigh day (d 0) and again 2 wk later (d 14). The temperature of the room was set at 29 °C for the first 3 d, and decreased by 0.3 °C daily until reaching a minimum set point of 24 °C.

The four dietary treatments were arranged as a 2 × 2 factorial (Tables 1 and 2), comparing two diet formulations and two phytase levels. Diet formulations consisted of a positive control (PC) meeting NRC (2012) recommendations vs. a negative control (NC) with 10% lower standardized ileal digestible (SID) lysine and added fat removed. A minimum ideal protein ratio (NRC, 2012) was maintained for all essential AA. The phytase levels were 0 vs. 2,500 FTU Quantum Blue 5G phytase/kg (QB5G; AB Vista Feed Ingredients, Marlborough, Wiltshire, UK). Inorganic P was included in all diets at levels that met the P requirement for nursery pigs (NRC, 2012); this ensured that the study

Table 1. Ingredient composition of experimental diets, experiment 1 (as-fed basis)

Phase	1		2		3		4	
	PC	NC	PC	NC	PC	NC	PC	NC
Treatment ^{1,2}								
Corn, %	35.75	38.74	44.19	47.35	57.35	60.65	67.20	70.35
Start premix ³ , %	50.00	50.00	31.25	31.25	13.89	13.89	—	—
Vita Plus piglet micro premix ³ , %	—	—	—	—	—	—	0.25	0.25
Soybean meal, %	10.60	8.85	20.81	18.90	23.90	21.80	27.90	25.95
Soybean oil, %	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
Monocalcium phosphate, %	0.85	0.85	0.75	0.75	1.25	1.25	1.35	1.35
Limestone, %	0.45	0.45	0.50	0.50	0.45	0.45	0.70	0.70
Aureomycin 50 (granular) ⁴ , %	0.40	0.40	0.40	0.40	—	—	—	—
Denagard 10 ⁵ , %	0.18	0.18	0.18	0.18	—	—	—	—
Mecadox ⁶ , %	—	—	—	—	1.00	1.00	0.50	0.50
Intellibond C2 ⁷ , %	—	—	—	—	—	—	0.04	0.04
Salt, %	0.25	0.25	0.25	0.25	0.35	0.35	0.35	0.35
L-lysine HCl, %	0.19	0.08	0.30	0.18	0.41	0.31	0.38	0.29
L-threonine, %	0.14	0.08	0.18	0.12	0.20	0.16	0.17	0.12
DL-methionine, %	0.19	0.13	0.20	0.12	0.20	0.14	0.16	0.10

¹The NC was achieved by reducing SID lysine by 10% with proportional lowering of all other essential AA, plus reducing added fat by 1 percentage point. Diet phases 1 through 3 were fed for 1 wk each whereas phase 4 was fed for 2 wk.

²Quantum Blue 5G Phytase (AB Vista Feed Ingredients; Marlborough, Wiltshire, UK) was added at 0.0063% to the PC and to the NC diets to produce the two dietary treatments containing 2,500 FTU/kg of phytase.

³Provided a minimum per kilogram of final diet: Fe, 132 mg; Mn, 54 mg; Cu, 23 mg; I, 0.4 mg; Se, 0.31 mg; Zn, 230 mg; vitamin A, 11.22 kIU; vitamin D, 2 kIU; vitamin E (total) 54 IU; vitamin K, 4 mg; niacin, 82 mg; riboflavin, 12 mg; pantothenic acid, 40 mg; vitamin B₁₂, 56 µg; pyridoxine, 5 mg; biotin, 0.27 mg; folic acid, 1 mg; choline, 1,220 mg.

⁴Zoetis, Inc., Florham Park, NJ; chlortetracycline 440.9 mg/kg in the diet.

⁵Elanco Animal Health, Greenfield, IN; tiamulin hydrogen fumarate 180.8 mg/kg in the diet.

⁶Phibro Animal Health Corporation, Teaneck, NJ; carbadox 220.5 mg/kg in the diet.

⁷Micronutrients, Indianapolis, IN; copper chloride.

evaluated the response to phytase unrelated to fulfilling the pigs' requirement for P. The phytase level was chosen based on the levels reported in the literature that improved performance when P was not deficient (Beaulieu et al., 2007; Zeng et al., 2014). During diet formulation, phytase was given

no credit for release of any nutrients other than P. Treatments were applied across a four-phase feeding program, with the first three phases fed for 1 wk each and the fourth phase fed for the final 2 wk of the 5-wk growth experiment. Diet samples were collected during manufacture, homogenized,

Table 2. Calculated nutrient composition of experimental diets, experiment 1 (as-fed basis)^{1,2}

Phase	1		2		3		4	
	PC	NC	PC	NC	PC	NC	PC	NC
Treatment								
ME, Mcal/kg	3.33	3.28	3.33	3.28	3.33	3.26	3.33	3.28
NE, Mcal/kg	2.39	2.35	2.42	2.39	2.45	2.41	2.49	2.46
Crude protein, %	23.01	22.21	21.26	20.38	19.69	18.79	18.27	17.42
SID lysine, %	1.44	1.31	1.38	1.24	1.30	1.17	1.18	1.06
SID methionine, %	0.54	0.47	0.52	0.45	0.48	0.41	0.42	0.36
SID total sulfur amino acids, %	0.84	0.83	0.86	0.78	0.81	0.73	0.74	0.67
SID threonine, %	0.94	0.86	0.90	0.81	0.85	0.78	0.77	0.69
SID tryptophan, %	0.26	0.25	0.24	0.23	0.22	0.21	0.20	0.19
Calcium, %	0.85	0.85	0.75	0.75	0.69	0.69	0.62	0.61
P, %	0.81	0.81	0.72	0.71	0.66	0.66	0.59	0.58
Standardized total tract digestible P, %	0.50	0.50	0.41	0.41	0.34	0.34	0.32	0.31
Ca:STTD P ratio	1.69	1.69	1.82	1.82	1.99	1.99	1.96	1.97

¹The NC was achieved by reducing SID lysine by 10% with proportional lowering of all other essential AA, plus reducing added fat by 1 percentage point; AA values were derived from internal database of ingredients which is based on prior AA assay.

²Diets formulated to contain 0 FTU phytase/kg were found upon assay to contain 0 FTU/kg.; all diets formulated to contain 2,500 FTU phytase/kg were found to contain an average of 3,091 FTU/kg.

subsampling, and stored at $-20\text{ }^{\circ}\text{C}$ for later chemical analysis.

Pigs were weighed on d 0, 7, 14, 21, 27, and 35 and feed intake was recorded for the same periods to facilitate determination of average daily gain (ADG), average daily feed intake (ADFI), and growth to feed ratio (G:F). Date of event and BW were recorded for all pigs removed from the study. Metabolizable and net energy conversion was calculated by multiplying the metabolizable energy (ME) or NE concentration in the diet by ADFI and then dividing the product by ADG.

Experiment 2. A total of 2,200 pigs (PIC Triumph \times PIC Camborough; PIC, Hendersonville, TN) with an average initial BW of 36.6 ± 0.3 kg were received at the Hanor Company Research Facility (White Hall, IL) and then sorted by sex into two identical, 50-pen, fully slatted barns equipped with a computerized feed delivery system (Big Dutchman, Inc., Holland, MI). Each pen was equipped with a four-space, dry self-feeder and a two-nipple hanging waterer. Pigs were blocked according to initial BW within gender and then randomly allotted to one of five dietary treatments within block. In accordance with commercial practice, both sexes received the same diets. Pens contained 19 to 24 pigs per pen; the number of pigs within pen was equalized within block. Upon achieving market weight, pigs were shipped to Triumph Foods (St. Joseph, MO) in four

shipments (cuts) over the course of 4 wk (i.e., one cut per week). Each shipment contained a similar number of pigs from each pen in order to balance harvest day across treatments.

The five dietary treatments (Tables 3 and 4) included a PC formulated to meet or exceed the pigs' requirements for all nutrients (NRC, 2012) and included 250 FTU QB5G phytase/kg (AB Vista Feed Ingredients); this level of phytase reflected a typical level of phytase added to pig diets to improve P release. The PC was included in the experiment to provide a baseline against which pig performance on the other treatments could be compared. The NC was formulated to be similar to the PC, except that it contained 12% less SID lysine and 0.75 percentage points less added fat; minimum ideal protein ratios of essential AA to lysine (NRC, 2012) were kept constant in all diets. The NC was included in the experiment to define performance when energy and AAs are reduced—this being the best model for revealing a response to increased energy and/or nutrient release when phytase is added at super-dosed levels, as predicted by some other researchers (Cowieson et al., 2011). In other words, if nutrients were all present at requirement (PC diet), and phytase increased nutrient availability, improvements in pig growth response may not be observed. Given the results of experiment 1, it was deemed unnecessary to supplement the PC diets with phytase; doing so would have increased

Table 3. Ingredient composition of experimental diets, experiment 2 (as-fed basis)

Phase	1		2		3		4		5	
	PC	NC								
Corn, %	56.70	57.69	63.02	63.94	64.36	63.94	67.44	68.37	68.73	69.62
Wheat middlings, %	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Soybean meal (47.5), %	22.50	22.50	16.25	16.25	15.00	15.00	12.00	12.00	10.75	10.75
Choice white grease, %	3.00	2.25	3.00	2.25	3.00	2.25	3.00	2.25	3.00	2.25
Vitamin trace mineral premix ² , %	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Monocalcium phosphate, %	0.580	0.580	0.560	0.560	0.570	0.560	0.580	0.580	0.590	0.590
Limestone, %	0.730	0.730	0.760	0.760	0.770	0.770	0.790	0.790	0.800	0.800
Salt, %	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400
L-lysine HCl, %	0.300	0.100	0.280	0.110	0.230	0.070	0.150	0.020	0.110	—
L-threonine, %	0.090	—	0.080	—	0.060	—	0.040	—	0.030	—
L-tryptophan, %	0.030	—	0.030	—	0.020	—	0.020	—	—	—
DL-methionine, %	0.015	—	0.050	—	0.020	—	—	—	—	—
Phytase ³ , %	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005

¹The NC was formulated to contain 12% less SID lysine with relative lowering of all other AA, plus 0.75 percentage points less added fat. "Diet phase 1 was fed for approximately 14 d, phase 2 through 4 were fed for approximately 21 d each, and phase 5 was the final phase provided to the pigs until they were harvested."

²Provided per kilogram of diet: Fe, 134 mg; Mn, 19 mg; Cu, 10 mg; I, 0.4 mg; Se, 0.30 mg; Zn, 80 mg; vitamin A, 7 kIU; vitamin D, 1 kIU; vitamin E 33 IU; vitamin K, 3 mg; niacin, 26 mg; riboflavin, 5 mg; pantothenic acid, 18 mg; vitamin B₁₂, 24 μ g.

³Quantum Blue 5G Phytase (AB Vista Feed Ingredients; Marlborough, Wiltshire, UK) was added to the NC at 0.020% to create the 1,000 FTU/kg dietary treatment, added at 0.035% to create the 1,750 FTU/kg dietary treatment, and added at 0.050% to create the 2,500 FTU/kg dietary treatment.

Table 4. Calculated nutrient composition of experimental diets, experiment 2 (as-fed basis)

Phase	1		2		3		4		5	
	PC	NC								
Treatment ¹										
ME, Mcal/kg	3.40	3.36	3.40	3.36	3.39	3.36	3.39	3.36	3.39	3.35
NE, Mcal/kg	2.57	2.53	2.60	2.56	2.60	2.57	2.62	2.58	2.62	2.59
Crude protein, %	18.48	18.24	15.91	15.74	15.33	15.21	14.03	13.95	14.03	13.95
SID lysine, %	1.05	0.89	0.87	0.74	0.80	0.68	0.66	0.56	0.60	0.51
SID methionine, %	0.32	0.24	0.26	0.21	0.22	0.21	0.19	0.19	0.19	0.19
SID total sulfur amino acids, %	0.59	0.51	0.49	0.45	0.45	0.44	0.41	0.41	0.39	0.40
SID threonine, %	0.64	0.55	0.54	0.47	0.51	0.45	0.44	0.41	0.42	0.39
SID tryptophan, %	0.21	0.19	0.18	0.15	0.16	0.15	0.14	0.13	0.12	0.12
Calcium, %	0.52	0.52	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
P, %	0.56	0.57	0.53	0.53	0.53	0.53	0.52	0.52	0.52	0.52
Standardized total tract digestible P, % ^{2,3}	0.31	0.32	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Ca:STTD P ratio	1.68	1.63	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67

¹The NC was formulated to contain 12% less SID lysine with relative lowering of all other AA, plus 0.75 percentage points less added fat; AA values derived from internal database of ingredients which is based on prior AA assay.

²Analyzed phytase levels averaged across phases within treatment: PC, 950 FTU phytase/kg; NC, 1,264 FTU phytase/kg; formulated 1,000 FTU phytase/kg, analyzed 1,500 FTU phytase/kg; formulated 1,750 FTU phytase/kg, analyzed 2,280 FTU phytase/kg; formulated 2,500 FTU phytase/kg, analyzed 2,500 FTU phytase/kg.

³All diets contained sufficient phytase to ensure the diets met or exceeded the pig's requirement for STTD P, according to [NRC \(2012\)](#). Quantum Blue 5G added at 0.005% of the diet provided at least 250 FTU phytase/kg which was assumed to release 0.08% STTD P. As the analyzed phytase levels were considerably higher than expected due to higher levels of phytase in the premix, there can be no question that the super-dosing effect is not due to meeting the pig's P requirement.

the number of experimental treatments and reduced the power of the experiment. Phytase was added at three so-called super-dosed levels—1,000, 1,750, and 2,500 FTU phytase/kg—to the NC to complete the five dietary treatments used in this experiment. The quantity of phytase chosen for this experiment was based on a range of levels that, if successful, would be considered economically practical. As in experiment 1, phytase was given no credit for release of any nutrients other than P when diets were formulated. This treatment regime was created to establish upper baseline performance for the group of pigs being evaluated (PC) along with the NC to confirm that energy and or nutrients were lowered sufficiently to impact performance. The three levels of phytase were used as it is not yet clear what level of phytase is required to achieve a “super-dose” response. Treatments were fed in five phases and phase changes corresponded with each weigh day.

Pens of pigs were weighed and feed disappearance were determined on d 0, 14, 35, 56, 77, 98, and at market, prior to each of the four cuts, to determine whole body ADG, ADFI, and G:F overall and by phase. Carcass gain was calculated by subtracting hot carcass weight (HCW) from the estimated initial carcass weight (initial BW × 0.74). The final dressing percentage was calculated by dividing the HCW by the live weight recorded on the farm at the time of harvest and multiplying by 100 (final dressing percentage = [HCW / market weight] ×

100). Live and carcass energy efficiency based on ME and NE were calculated by whole body ADG and carcass ADG dividing the products of ADFI by diet ME or NE content, respectively.

Chemical Analysis

Feed samples from both experiments were analyzed for phytase content by method 2000.12 ([AOAC Int., 2015](#)) by Eurofins Nutrition Analysis Center (Des Moines, IA).

Data Analysis

In both experiments, pen was the experimental unit, treatment was a fixed effect, and block was a random effect. Data were checked for normality using PROC UNIVARIATE of SAS 9.4 (SAS Institute, Cary, NC). Main effects and interactions were considered significant if $P \leq 0.05$ and trends if $P > 0.05$ or $P \leq 0.10$.

In experiment 1, data were analyzed according to the 2 × 2 factorial arrangement of treatments, using PROC MIXED of SAS 9.4 (SAS Institute), considering both main effects and their interaction.

In experiment 2, data were analyzed using PROC MIXED of SAS 9.4 (SAS Institute) with polynomial contrasts comparing the PC treatment vs. the NC treatment, and the average of the three super-dosed levels of phytase against the NC treatment,

as there were no differences among phytase levels. If there was a significant difference between the NC and the mean of the three super-dosed phytase treatments, linear and quadratic contrasts were then applied. If there was a significant sex \times treatment interaction, polynomial contrasts were used to determine whether the nature of the interaction was a result of the sexes responding to super-dosing phytase differently, or if the response was due to the differences in response between the PC and the NC relative to sex.

RESULTS

In experiment 1, there were no significant interactions between diet composition and phytase level, so only main effects will be reported. The PC treatment resulted in heavier final weights, faster growth rate, and more efficient utilization of feed and of metabolizable and net energy, as compared to the NC (Table 5; $P < 0.05$); there was no effect on ADFI ($P > 0.10$). This confirmed that the experimental model—a positive fully balanced control diet vs. a reduced energy and nutrient diet—worked, as the NC treatment was clearly shown to be deficient.

Super-dosed phytase supported faster gains and greater feed and energy efficiency ($P < 0.05$). There was no effect on ADFI ($P > 0.10$). Of particular interest, there were no interactions between diet and phytase for overall ADG, ADFI, G:F, or the efficiency of energy utilization ($P > 0.10$), indicating that a deficient diet was not necessary to evoke a response to super-dosing, nor was there greater impact when the diet was deficient.

In the grow-finish experiment (experiment 2), the PC resulted in heavier final BW as well as faster and more efficient growth compared with the NC ($P < 0.001$; Table 6). This treatment also improved the efficiency with which the pigs used ME and NE for growth ($P < 0.001$). Once again, the experimental model worked, as the expected reduction in growth was observed in the NC pigs.

Pigs consuming the super-dosed phytase tended to use feed and dietary energy more efficiently than pigs consuming the NC treatment ($P < 0.10$). Super-dosing did not result in an improvement in final BW, ADG, or ADFI ($P > 0.10$).

The PC diet supported heavier HCWs and a higher dressing percentage ($P < 0.05$; Table 7). Pigs consuming the super-dosing treatments had higher HCW than pigs consuming the NC ($P < 0.05$). When calculating performance on a carcass basis, the results are somewhat different than are observed using the more conventional whole body basis. On a carcass basis, the PC diet supported faster and more efficient gain ($P < 0.001$). However, unlike data calculated on a whole body basis, super-dosing phytase improved carcass-based feed and energy efficiency ($P < 0.05$) and tended to improve carcass ADG ($P < 0.10$). Gilts on the PC were more efficient at converting feed and energy to gain than barrows on the PC, although the opposite is true for the NC and three super-dosing treatments, with barrows being more efficient than gilts (data not shown, $P < 0.05$).

The results of diet assays revealed higher than expected dietary phytase levels, especially in experiment 2. In experiment 1, the zero phytase treatment was confirmed to contain no phytase. However, the super-dosed treatment contained 3,091 FTU phytase/kg, compared to an expected

Table 5. Main effects of the impact of diet adequacy and super-dosed phytase on nursery pig growth performance, experiment 1¹

Item	Diet ²		Phytase, FTU/kg			Diet	P values	
	PC	NC	0	2,500	SEM		Phytase	Diet \times Phytase
Initial BW, kg	6.26	6.27	6.27	6.26	0.285	0.094	0.693	0.629
Final BW, kg	22.44	21.68	21.89	22.23	0.501	0.002	0.144	0.342
ADG, kg/d	0.48	0.46	0.46	0.47	0.007	0.001	0.039	0.450
ADFI, kg/d	0.67	0.67	0.67	0.66	0.010	0.341	0.172	0.563
G:F	0.72	0.68	0.68	0.71	0.004	<0.001	<0.001	0.558
Energy efficiency, kg gain/Mcal ME or NE ³								
ME	0.22	0.21	0.21	0.22	0.001	0.001	<0.001	0.623
NE	0.29	0.30	0.29	0.30	0.002	<0.001	<0.001	0.233

¹Data are least square means; $n = 10$ pens per treatment with 10 pigs per pen, totaling 440 pigs; sexes were not split but were even across treatments; 35-d trial.

²The NC was achieved by reducing SID lysine by 10% with proportional lowering of all other essential AA, plus reducing added fat by 1 percentage point.

³Energy efficiency calculated as: $ADG / (ME \text{ or } NE \text{ of diet} \times ADFI)$.

Table 6. Impact of diet energy and nutrient levels and super-dosed phytase on whole body grow-finish growth performance, experiment 2¹

Item	Treatment ²					SEM	P value	
	PC	NC	1,000 FTU phytase/kg	1,750 FTU phytase/kg	2,500 FTU phytase/kg		PC vs. NC ³	NC vs. SD ⁴
Initial BW, kg	36.77	36.56	36.57	36.51	36.50	0.412	0.160	0.722
Final BW, kg	125.26	120.12	121.86	121.39	122.04	0.746	<0.001	0.217
ADG, kg/d	1.011	0.960	0.974	0.961	0.972	0.007	<0.001	0.189
ADFI, kg/d	2.867	2.853	2.878	2.827	2.857	0.021	0.577	0.952
G:F ⁵	0.353	0.336	0.339	0.340	0.340	0.002	<0.001	0.084
Energy efficiency, kg gain/Mcal ME or NE ⁶								
ME ⁵	0.104	0.100	0.101	0.101	0.101	0.0005	<0.001	0.083
NE ⁵	0.136	0.131	0.132	0.133	0.133	0.0006	<0.001	0.083

¹Data are least square means; $n = 20$ pens per treatment total with 19 to 24 pigs per pen, split by sex with 50 pens of gilts and 50 pens of barrows; 98-d trial.

²The NC was formulated to contain 12% less SID lysine with relative lowering of all other AA, plus 0.75 percentage points less added fat. Both PC and NC contained 250 FTU phytase/kg.

³Linear contrast of PC vs. NC.

⁴Comparison of NC vs. mean of three super-dosing treatments.

⁵Data not shown: sex \times treatment, $P < 0.001$.

⁶Energy efficiency calculated as: $ADG / (ME \text{ or } NE \text{ of diet } \times ADFI)$.

2,500 FTU/kg. In experiment 2, the control diets were formulated to contain 250 FTU phytase/kg but in reality the PC contained 950 FTU/kg and the NC contained 1,264 FTU/kg. The super-dosed diets contained 1,500, 2,280, and 2,500 FTU/kg for the 1,000, 1,750, and 2,500 FTU/kg treatments, respectively. It was assumed that the phytase premix

contained more phytase activity than expected, thus explaining the deviations.

DISCUSSION

In both experiments, the fundamental platform for evaluating super-dosing phytase was to include the enzyme in a diet that was lower in energy and

Table 7. Impact of diet energy and nutrient levels on carcass-based grow-finish growth performance, experiment 2¹

Item	Treatment ²					SEM	P value	
	PC	NC	1,000 FTU phytase/kg	1,750 FTU phytase/kg	2,500 FTU phytase/kg		PC vs. NC ³	NC vs. SD ⁴
HCW, kg ⁵	92.96	89.01	90.03	89.81	89.90	0.652	<0.001	0.043
Dressing percent, % ^{6,7}	74.54	74.03	74.22	74.00	73.76	0.172	0.032	0.832
ADG, kg ⁷	0.738	0.695	0.708	0.702	0.704	0.005	<0.001	0.067
G:F ⁸	0.270	0.256	0.257	0.260	0.258	0.001	<0.001	0.040
Full value pig, % ^{7,9}	96.35	97.40	97.74	96.60	96.35	0.869	0.379	0.605
Energy efficiency, kg gain/Mcal ME or NE ¹⁰								
ME ⁸	0.076	0.072	0.073	0.074	0.073	0.0004	<0.001	0.033
NE ⁸	0.099	0.095	0.096	0.097	0.096	0.0005	<0.001	0.033

¹Data are least square means; $n = 20$ pens per treatment with 19 to 24 pigs per pen, split by sex with 50 pens of gilts and 50 pens of barrows; 98-d trial.

²The NC was formulated to contain 12% less SID lysine with relative lowering of all other AA, plus 0.75 percentage points less added fat. Both PC and NC contained 250 FTU phytase/kg.

³Linear contrast of PC vs. NC.

⁴Comparison of NC vs. mean of three super-dosing treatments.

⁵Data not shown: sex \times treatment, $P < 0.10$.

⁶Dressing percentage: $(HCW / \text{live weight}) \times 100$.

⁷Data not shown: sex \times treatment, $P > 0.10$.

⁸Data not shown: sex \times treatment, $P < 0.05$.

⁹Full value pig: $(\text{number of pigs put on trial} - \text{total dead or removed}) / \text{number of pigs put on trial} \times 100$.

¹⁰Energy efficiency calculated as: $ADG / (ME \text{ or } NE \text{ of diet } \times ADFI)$.

deficient in AA, but sufficient in P. The hypothesis was that super-dosed phytase acts by enhancing energy and/or nutrient availability (Olsen et al., 2018). If this is the case, the response to phytase would be more likely to be observed when the diets are limiting relative to the pigs' requirements. However, the results of experiment 1 revealed that the response to super-dosed phytase was no greater in a marginally deficient diet than one which is fully balanced for all nutrients. The marginally deficient diet was still used in experiment 2, but there was clearly no need to use the factorial design. The need for additional diets, and therefore fewer observations per treatment, when applying the factorial design was considered to be less important than evaluating multiple levels of phytase at super-dosed levels. In this way, experiment 1 and 2 complement each other.

Other researchers have reported results similar to ours in nursery pigs. Biehl and Baker (1996) fed diets limiting or not limiting in AA and with or without 1,200 FTU phytase/kg. They reported that phytase improved ADG, regardless of AA levels, similar to the outcome of the present experiment 1, except that they reported only an improvement in G:F in pigs fed diets limiting in AA. Their slightly different outcome may be attributed to their lower levels of "super-dosing." Research by Kies et al. (2006) supports this interpretation, as they reported improved feed efficiency as phytase increased from 0 to 1,500 to 15,000 FTU/kg.

The oft-stated explanation that improved AA digestibility is the mechanism for super-dosing phytase may be more supposition than fact as the improvement in AA digestibility may not apply uniformly across all diets, but rather be restricted to low-protein circumstances (Adeola and Sands, 2003). Bohlke et al. (2005) observed a greater digestibility of some AA (Arg, Ile, Lys, Phe, Thr, Val, Asp, and Gly) when pigs were fed a low-phytate corn diet compared to a standard corn diet, with corn as the only protein source. The authors attributed the differences in digestibility to phytate-AA interactions.

It perhaps makes more sense to attribute the improved performance to increased availability of energy. It is possible in experiment 1 that the PC diet, as well as the NC diet, contained too low a concentration of energy to support maximal performance in nursery pigs. It is generally accepted that pig growth in the nursery phase is restricted by an insufficient energy supply, simply due to physical gut capacity (Patience et al., 1995). In other words, young pigs cannot consume sufficient feed

to supply enough energy to support their full genotypic potential for growth. If phytase increased energy availability, the pigs would be able to grow faster. The absence of an interaction between the PC and the NC diets in experiment 1, combined with the clear response in feed efficiency, would support this explanation.

In experiment 2, super-dosing phytase resulted in overall trends for improvements in whole body efficiency of energy and feed utilization. Interestingly, when the data were calculated on a carcass basis, pigs super-dosed with phytase showed improvement in feed and feed energy efficiency and a tendency for improved growth rate. The greater statistical significance using carcass data is probably due to the fact that the benefit of phytase on ADG was greater on a carcass basis than on a whole body basis, and the variation in the energy efficiency data was slightly reduced. However, there is no obvious biological explanation why the carcass-based data showed a greater response. These results have two implications. First, super-dosing phytase improved carcass-based feed and feed energy efficiency when energy and AA were limiting. Second, the value of evaluating growth performance based on carcass outcomes was reaffirmed; this is particularly critical when pigs are sold on a carcass as opposed to live weight basis (Weber et al., 2015), which is the predominant method in many parts of the world. It should be noted that super-dosed phytase did not improve carcass yield, which raises questions about the different response observed on a whole body basis vs. carcass basis in this experiment.

The growth response to super-dosed phytase in grow-finish pigs has not been observed consistently. Braña et al. (2006) reported a positive response but Flohr et al. (2014) did not. The difference in outcome may be the consequence of basal diet formulation. In the study by Braña et al. (2006), the basal diet was limiting in available P whereas that by Flohr et al. (2014), similar to the present experiment 2, was properly balanced for P. It should be noted that the diets used by Flohr et al. (2014) differed from the present experiment 2 in that they were not deficient in energy or AA.

It is possible that another mechanism is involved, such as removing the antinutrient effects of phytate and/or generating the release of other limiting components of the diet; in this instance, inositol could be a candidate (Holloway et al., 2016). Furthermore, if phytate is interacting with gastrointestinal enzymes, such as α -amylase (Deshpande and Cheryan, 1984), super-dosing phytase may mitigate this interaction, leading to an increase in starch

digestion, resulting in increased energy digestibility, and improved pig performance. However, most starch is digested by the end of the small intestine, so an improvement in starch digestibility is probably of limited value to the pig (Wilfart et al., 2007). If there is a benefit, it would likely be more evident in younger nursery pigs, due to the decreased enzyme activity and drastic dietary substrate change that occurs at weaning. Thus, even a small improvement in the digestibility of starch or other nutrient would result in improved growth performance because of the newly weaned pig inherently having limited digestive capacity (Patience et al., 1995; Jensen et al., 1997). This may explain the greater response in gain, feed, and feed energy efficiency in experiment 1 compared to experiment 2.

Another possible mechanism is the release of lower derivative inositols or free myo-inositol (Holloway et al., 2016). Cowieson et al. (2013) reported improvements in growth performance and insulin levels in broilers supplemented with myo-inositol, lending support to this theory. There do not appear to be corresponding data in swine. Inositol is involved in the phosphoinositide family of lipids. Phosphoinositides are stored in the plasma membrane and are a source of lower derivative inositols, which are used as secondary messengers in insulin signaling and intracellular Ca signaling (Croze and Soulage, 2013). Inositol derivatives are synthesized within the animal de novo (Jiao et al., 2015). Thus, by increasing dietary inositol, there may be less need for inositol synthesis by the animal, or perhaps there is an increase in inositol usage, which is contributing to the observed improvements in growth performance.

It should be noted that the actual levels of phytase in the diets were above expectation. In experiment 1, 2,500 FTU phytase/kg was compared to a diet with no added phytase, so the fact that the actual phytase level was above 3,000 FTU/kg probably had little impact on the outcome. However, in experiment 2, the lowest levels of phytase, found in each of the control treatments, were formulated to contain 250 FTU/kg but actually averaged about 1,100 FTU/kg. Thus, the lowest phytase level was approaching “super-dosing” levels; as such, the growth response observed in experiment 2 may have been greater had the control actually contained only 250 FTU/kg as planned. This underscores the need to pre-assay all enzyme sources, and all final diets, in studies investigating enzyme responses. This example is not the only one in the literature where assayed levels of phytase were considerably higher than expected (Jones et al., 2010); however, actual phytase levels

were not always determined or reported (Nortey et al., 2015; Guggenbuhl, et al., 2016).

In conclusion, super-dosing phytase improved nursery pig growth performance, increasing both ADG and feed efficiency. These improvements were not affected by basal diet energy or nutrient concentration. Super-dosing phytase also improved carcass-based growth performance in pigs in the grow-out phase of production, with improvements in both feed efficiency and feed energy efficiency. These improvements due to super-dosing were less apparent on a live weight basis, demonstrating the value in considering grow-finish data on both a carcass basis and a live weight basis. The benefits to the use of super-dosing were of greater magnitude in the nursery than in the grow-finish phase of production, but were nonetheless present in both experiments.

Conflict of interest statement. None declared.

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