EFFECT OF PHYTASE ON PERFORMANCE AND BONE MINERALIZATION IN GROWING PIGS FED A DEFICIENT P DIET

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INTRODUCTION

Phytase is an exogenous enzyme that catalyses the hydrolysis of phytate releasing phosphorus (P) and other chelated minerals such as zinc, magnesium or calcium for use by the animal (Rutherfurd et al. 2012). In pigs, phytase increases P digestibility and absorption (Selle et al. 2003). This allows for a reduction of dietary P, thereby reducing the cost of the formula and P excretion into the environment. The amount of P released by phytase is dependent on the level of dietary phytate in the diet.

OBJECTIVE

To determine if phytase can be added to replace 0.17% digestible P in a diet containing 0.23% level of dietary phytate-P, and its influence on growth performance, and bone characteristics in growing pigs.

MATERIAL & METHODS

Phytase: Quantum Blue (*E.coli* derived, intrinsic thermo-tolerant 6-phytase produced by *Trichoderma reesei*).
Diets: Corn (40%), pre-treated wheat (35%) and soybean meal (18%) based diets, fed *ad libitum* in pelleted form.
Experimental design: 5 treatments, 9 replicates each, 4 pigs per pen.
Treatments:

T1- Positive control (PC)
T2- Negative control (NC)
T3- NC + 300 FTU/kg of phytase (NC+300)
T4- NC+600 FTU/kg of phytase (NC+600)
T5- NC+1200 FTU/kg of phytase (NC+1200)

Table 1. Dietary P and Ca level (%) of the experimental diets

Diet	P total	P digestible	Ca
Positive Control (PC)	0.58	0.28	0.70
Negative Control (NC)*	0.35	0.11	0.49

*added with phytase at 300 (NC+300), 600 (NC+600) or 1200 FTU/kg feed (NC+1200)

Animals: 180 male and female Pietrain*(Landrace*Large White) pigs of 20 kg liveweight.
Parameters: Growth performance, and feed efficiency after 42 days of trial.
At the end, 1 pig / pen was euthanized and metatarsal bones collected for bone strength, and bone ash and mineral analysis.
Statistical evaluation: GLM procedure of SAS, and means were compared using a Student-Newman-Keuls test.

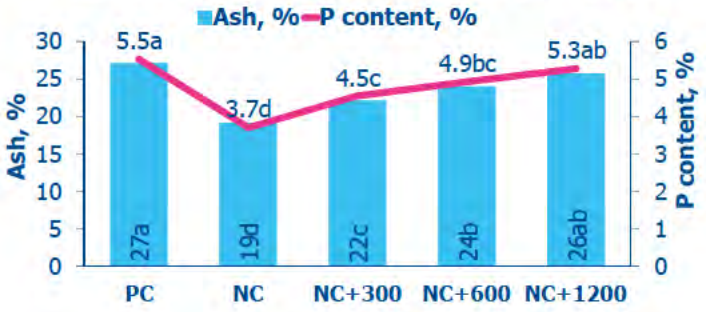
RESULTS & DISCUSSION

Table 2. Growth performance and bone strength of growing pigs.

Diet	BW, kg	ADG, g/d	FCR	Strength, N
T1-PC	47.2a	644a	1.99b	548a
T2-NC	40.6b	488b	2.09a	253c
T3-QB300	46.9a	634a	1.99b	418b
T4-QB600	45.9a	615a	2.04b	411b
T5-QB1200	46.7a	633a	1.99b	521a
SEM	*0.69*	*16.3*	*0.03*	*24.2*

a-c Means followed by different superscripts within columns are statistically different (P≤0.05)

Figure 1. Bone ash and P content (%) of growing pigs.



- Regardless the dose used, phytase inclusion improved weight gain, feed efficiency, and bone strength (P<0.01) relatively to the NC diet.
- No differences were observed between the PC diet and those containing phytase.
- Metatarsals of pigs fed PC and NC diets showed the highest and lowest bone strength (P<0.001), respectively.

- Bone ash and P contents were reduced (P<0.01) on pigs fed the NC relatively to PC diet.
- Bone ash and P content respond proportionally to increasing levels of phytase supplementation.
- The highest phytase dosage was able to restore bone ash and P content to similar levels as the PC.

CONCLUSION

- ❑ Reduction of dietary P and Ca impaired performance and bone characteristics of growing pigs.
- ❑ Phytase supplementation, regardless of dose level, restored performance to similar levels of PC fed pigs.
- ❑ Bone mineralization and bone strength were improved in a linear fashion by phytase addition while the highest phytase dosage was able to restore bone parameters to similar levels as PC fed pigs.
- ❑ These results highlight the ability of the tested phytase to breakdown phytate-P increasing the availability of dietary P.

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資料3

Performance and bone characteristics of growing pigs fed diets marginally deficient in available phosphorus and a novel microbial phytase

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Santos, T. T., Walk, C. L., Wilcock, P., Cordero, G. and Chewning, J. 2014. **Performance and bone characteristics of growing pigs fed diets marginally deficient in available phosphorus and a novel microbial phytase**. Can. J. Anim. Sci. **94**: 493–497. The efficacy of a novel microbial 6-phytase on pig performance, bone breaking strength, and bone ash was evaluated. Pigs were allotted to one of six diets with 12 replicate pens/treatment and five pigs/pen. Diets consisted of a positive control (PC), negative control (NC) 1 with Ca and available phosphorus (avP) reduction by 0.11 and 0.10%, NC 1 plus 250 FTU kg^{-1} phytase, NC 2 with Ca and avP reduction by 0.16 and 0.15%, NC 2 plus 500 and 2,000 FTU kg^{-1} phytase. On day 43, one pig per pen was euthanized and the 4th metacarpal was obtained to determine bone parameters. Average daily gain (ADG) was higher ($P < 0.05$) in pigs fed NC 2 plus 500 or 2000 FTU kg^{-1} compared with PC. NC 2 with 2000 FTU kg^{-1} improved ($P < 0.05$) G:F compared with PC. NC 1 or NC 2 had reduced ($P < 0.05$) bone ash weight and NC 2 had reduced ($P < 0.05$) bone breaking strength compared with PC. Phytase supplementation in NC 1 or NC 2 improved bone ash weight compared with PC. Bone breaking strength or bone ash weight was more sensitive to low dietary Ca and avP than bone ash percent. Phytase supplementation at 2000 FTU kg^{-1} improved G:F beyond that of the PC.

Key words: Bone breaking strength, bone ash, performance, phytase, pigs

Santos, T. T., Walk, C. L., Wilcock, P., Cordero, G. et Chewning, J. 2014. **Performance de croissance et caractéristiques des os des porcs nourrisd'aliments légèrement carencés en phosphore assimilable et incluant une nouvelle phytase microbienne**. Can. J. Anim. Sci. **94**: 493–497. L'efficacité d'une nouvelle 6-phytase microbienne a été évaluée selon les performances de croissance des porcs, de résistance des os à la rupture (BBS – « bone breaking strength »), et de la teneur en cendre d'os chez les porcs. Les porcs ont été attribués à 1 de 6 diètes (traitements), avec 12 enclos/traitement et 5 porcs/enclos. Les traitements étaient un témoin positif (PC – « positive control »), un témoin négatif 1 (NC 1 – « negative control 1 ») ayant un taux de Ca et de P assimilable réduits de 0,11 et 0,10 % respectivement, NC 1 plus 250 FTU kg^{-1} de phytase, NC 2 ayant un taux de Ca et de P assimilable réduits de 0,16 et 0,15 % respectivement, NC 2 plus 500 ou 2000 FTU kg^{-1} de phytase. Au jour 43, 1 porc/enclos a été euthanasié et le quatrième métacarpe prélevé pour déterminer les paramètres osseux. Le gain moyen quotidien (ADG – « average daily gain ») était plus élevé ($P < 0{,}05$) chez les porcs nourris à la diète NC 2 plus 500 ou 2000 FTU kg^{-1} par rapport à la diète PC. La diète NC 2 plus 2000 FTU kg^{-1} a permis d'améliorer ($P < 0{,}05$) le ratio G:F par rapport à la diète PC. Les diètes NC 1 et NC 2 ont réduit ($P < 0{,}05$) le poids de la cendre d'os et la diète NC 2 a réduit ($P < 0{,}05$) la BBS par rapport à la diète PC. L'ajout de phytase dans les diètes NC 1 ou NC 2 améliore le poids de la cendre d'os par rapport à la diète PC. La BBS et le poids de la cendre d'os étaient plus sensibles aux faibles teneurs en Ca alimentaire et en P assimilable que le pourcentage de cendre d'os. L'ajout de la phytase, à raison de 2000 FTU kg^{-1}, améliore le ratio G:F au-delà de celui de la diète PC.

Mots clés: Résistance des os à la rupture, cendre d'os, performance, phytase, porcs

Phytate (salt of myo-inositol hexaphosphate) is the main source of P in vegetable ingredients used in animal feeds (Ravindran et al. 1994) and is poorly digested by monogastric animals. Phytase catalyses the hydrolysis of phytate and increases P digestibility and absorption in pigs (Selle et al. 2003). This allows for a reduction in the total P concentration in the diet, thereby reducing the cost of the diet and P excretion into the environment. In addition to being a low available source of P, phytate is a significant anti-nutrient in pig diets. Phytate reduced mineral (Schlegel et al. 2010), protein, and energy digestibility (Liao et al. 2005) and significantly reduced young pig growth performance (Woyengo et al. 2012).

The effect of superdoses of a novel microbial phytase to reduce the anti-nutritional effects of phytate has been previously reported in young piglets fed nutritionally adequate diets (Walk et al. 2013). In addition, previous authors have reported improvements in growth

Abbreviations: **ADG**, average daily gain; **avP**, available phosphorus; **BW**, body weight; **FTU**, phytase unit; **G:F**, gain to feed ratio; **NC**, negative control; **PC**, positive control

performance and Ca and P digestibility of growing pigs fed 250 and 500 FTU kg^{-1} of the same novel microbial phytase in reduced Ca and available P (avP) diets (Kuhn and Manner, 2012). Therefore, the objective of the present trial was to evaluate the efficacy of the novel microbial phytase on growth performance and bone parameters using two levels of avP and Ca and three doses of phytase (250, 500 and 2000 FTU kg^{-1}) in growing pigs between 23 and 55 kg.

MATERIALS AND METHODS

The trial was conducted according to the US government principles for the utilization and care of vertebrate animals used in testing, research, and training at Swine Research Services, Inc. in Springdale, Arkansas.

Approximately 6 wk post-weaning, 360 castrated male and female PIC pigs (22.7±8 kg) were randomly distributed into six dietary treatments according to body weight (BW) and gender. Each treatment contained six pens of castrated males and six pens of females to total 12 replicate pens per treatment with five pigs per pen. Pigs had ad libitum access to pelleted feed and water for the duration of the trial. Experimental diets were fed in a two-phase feeding program. Phase 1 was fed from 23 to 38 kg (day 0 to day 21) and phase 2 was fed from 38 to 55 kg (day 22 to day 43). Treatments in each phase consisted of a positive control (PC) diet formulated to meet or exceed nutritional requirements for each feeding phase (National Research Council 1998), a negative control (NC) 1 diet with a 0.11 and 0.10% reduction of Ca and avP, respectively, from the PC, NC 1 plus 250 FTU kg^{-1} phytase, a NC 2 diet with a 0.16 and 0.15% reduction of Ca and avP, respectively, from the PC, NC 2 plus 500 FTU kg^{-1} phytase, and NC 2 plus 2000 FTU kg^{-1} phytase. The phytase used was a novel, intrinsically thermostable, *Escherichia* coli 6-phytase expressed in *Trichoderma reesei* and contained a declared activity of 5000 FTU g^{-1} (Quantum Blue, AB Vista Feed Ingredients, Marlborough, UK). Feed samples were collected and analysed for phytase recovery according to methods of Engelen et al. (2001), where 1 phytase unit is defined as the amount of enzyme required to release 1 μmol of inorganic P min^{-1} from sodium phytate at pH 5.5 and 60°C. The ingredient composition and nutrient analyses of the experimental diets for phase 1 and phytase 2 are presented in Tables 1 and 2, respectively. Prior to the start of the experiment, a sample of each diet was collected and analyzed for CP, ether extract, Ca and total P (Association of Official Analytical Chemists 1990).

Pig BW and feed disappearance were measured at the beginning of the experiment (day 0), at the end of phase 1 (day 21), and at the end of the experiment (day 43) to calculate average daily gain (ADG), average daily feed intake, and gain to feed ratio (G:F). On day 43, one pig of average BW/pen was selected and euthanized for determination of bone breaking strength and ash percent. The right front foot was collected and the 4th metacarpal was dissected, placed into labeled 50-mL Falcon tubes, and stored at −25°C until further analyses. Bone breaking strength was determined after thawing using a three-point bend rig with a load cell capacity of 250 kg and cross-head speed of 100 mm min^{-1} (HD 250 Texture Machine, Scarsdale, NY). After determining breaking strength, any pieces from the individual bones were collected, wrapped in cheese cloth, and dried in an oven at 100°C for 24 h. Fat was extracted from the metacarpals using a 48 h Soxhlet extraction in ethyl alcohol followed by a 48-h extraction with diethyl ether. The bones were then dried at 110°C in an oven for 24 h and weighed. To determine bone ash percent, the dry, de-fatted metacarpals were ashed in a muffle furnace at 560°C for 48 h.

Table 1. Nutrient and chemical composition of experimental diets (as-fed basis; phase 1)

Item	Positive control	Negative control 1[z]	Negative control 2[y]
Ingredient (%)			
Corn	65.78	66.93	67.38
Soybean meal	28.40	28.30	28.30
Pork fat	2.25	1.80	1.60
Salt	0.40	0.40	0.40
L-Lys HCl	0.30	0.30	0.30
DL-Met	0.12	0.12	0.12
L-Thr	0.10	0.10	0.10
Dicalcium phosphate	1.10	0.60	0.40
Limestone	1.05	0.95	0.90
Vitamin-mineral premix[x]	0.50	0.50	0.50
Calculated analysis			
AME (kcal kg^{-1})	3,300	3,300	3,300
CP (%)	18.50	18.50	18.50
Digestible lysine (%)	1.10	1.10	1.10
Ca (%)	0.71	0.60	0.55
Total P (%)	0.60	0.50	0.45
Available P (%)	0.32	0.22	0.17
Analysed composition			
DM (%)	87.50	87.00	87.90
Ether extract (%)	4.08	3.83	3.63
CP (%)	18.30	19.30	18.90
Ca (%)	0.78	0.66	0.62
Total P (%)	0.59	0.48	0.46

[z]An additional diet identical to negative control 1 was formulated to contain phytase at the expense of corn at 0.005%. This level of phytase was equivalent to 250 FTU kg^{-1} of the diet. The phytase used was Quantum Blue (AB Vista Feed Ingredients, Marlborough, UK) and had an expected activity of 5000 FTU kg^{-1}.

[y]Two additional diets identical to negative control 2 were formulated to contain phytase at the expense of corn at 0.010 or 0.040%. This level of phytase was equivalent to 500 or 2000 FTU kg^{-1} of the diet, respectively. The phytase used was Quantum Blue (AB Vista Feed Ingredients, Marlborough, UK) and had an expected activity of 5000 FTU kg^{-1}.

[x]Supplied per kilogram of diet: vitamin A (retinyl acetate), 2500 IU; vitamin D (cholecalciferol), 600 IU; vitamin E (α-tocopherol acetate), 11 IU; vitamin K (menadione dimethylpiridinol bisulfate), 11 mg; riboflavin, 3 mg; pantothenic acid, 7 mg; niacin, 9 mg; thiamine, 12 mg; pyridoxine, 20 mg; vitamin B_{12}, 0.10 μg; Zn (ZnO), 120 mg; Fe ($FeSO_4.H_2O$), 100 mg; Mn (MnO), 20 mg; Cu ($CuSO_4.5H_2O$), 30 mg; I (KI), 1 mg and Se (Na_2SeO_3) 40 μg.